FLOWER PIGMENTS IN YELLOW *DENDROBIUM* SPECIES AND HYBRIDS

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ABSTRACT

Carotenoids and chlorophylls in yellow petals of Dendrobium species and hybrids were examined by high-performance liquid chromatography (HPLC). Six carotenoids, neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, and β-carotene, and chlorophyll a and b were identified. Lutein, zeaxanthin, and β-carotene were the major pigments responsible for yellow flower color in several dendrobiums. Color was affected by the numbers and concentrations of pigments.

The degradation of flavonoids, carotenoids, and chlorophylls in the flowers at different stages of maturity within a raceme was examined for three progenies, K528 (pale yellow), K637 (white-purple), and K650 (bright yellow). The amounts of carotenoids and chlorophylls in crosses K528 and K637 decreased rapidly after blooming, while cross K650 maintained a high carotenoid content in all stages of maturity. Flavonol content in all three crosses did not show any change over time.

Carotenoid and chlorophyll changes in growth and development from the bud stage to flower maturity were determined qualitatively and quantitatively in crosses K528 and K637. All 6 carotenoids and 2 chlorophylls in both crosses declined continually from the bud stage to 2-4 weeks after blooming.

The inheritance of yellow flower color in Dendrobium appears to be complex, since at least 6 carotenoids and chlorophyll a and b have been shown to co-exist. Degradation of yellow pigments is not uncommon, and polyploidy further complicates the analysis of color inheritance. Since both carotenoid and chlorophyll concentrations in hybrids often fall between those of the parents, it appears that yellow color is mostly quantitatively inherited although interactions with modifying genes can be significant.

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I. INTRODUCTION

Dendrobium is one of the largest genera in the orchid family. It consists of about 1,400 species distributed in tropical Asia and Australasia (Dressler, 1981). This large genus has been subdivided into 41 sections based on floral and vegetative characteristics (Schlechter, 1912).

Dendrobiums are popular in Hawaii because of the diversity in plant form and floral characteristics. They have been widely used for landscaping, potted plants, corsages, and flower arrangements. They are commercially cultivated for potted plant and cut flower sales. In 1983, the wholesale values for potted dendrobiums and cut dendrobiums were $1,032,000 and $774,000, respectively (Davis and Martin, 1984). The production of dendrobium cut racemes increased 25% in Hawaii during the period 1972-1980 (Leonhardt *et al.*, 1981).

The colors of dendrobium flowers vary over a wide range of visible spectra (violet to red) and depend on three major groups of pigments: flavonoids, carotenoids, and chlorophylls. Most commercial dendrobium cultivars fall into the flower color categories of white, purple, and purple-violet. Good clear yellow cut flower cultivars are seldom available in the market. Most of them are not pure yellow, but mixed with green. This suggests that

carotenoids and chlorophylls are found in the yellow flower. Carotenoids and chlorophylls were observed in many orchid genera other than Dendrobium (Dueker and Arditti, 1968; Arditti and Ernst, 1969; Harper, 1972b; Lowry and Keong, 1973; Griesbach, 1983 and 1984). However, no studies of methodology on extraction and identification of carotenoids and chlorophylls have been reported.

A breeding program for improving yellow dendrobium cultivars was initiated at the University of Hawaii in 1971. Over 100 crosses have been made to date. Most of the progenies are polyploids and have ancestors from sections Ceratobium and Phalaenanthe. Observations to date suggest that lavender and purple are dominant to white. A problem encountered is that yellow flower color often fades after blooming. Due to the relatively long life cycles of 4-5 years, genetic studies in Dendrobium are still in its infancy compared to other crops such as petunia and corn.

The purpose of this study is to develop a methodology for identification of carotenoids and chlorophylls in flower tissue, to examine the degradation of flower pigments, and to elucidate the inheritance of yellow flower pigments. Such knowledge should aid in the breeding program for the development of improved yellow Dendrobium cultivars.

II. REVIEW OF LITERATURE

2.1 Yellow Flower Pigments

Carotenoids and flavonoids are the 2 groups of pigments determining yellow color of flowers. Carotenoids, accumulating in chloroplasts and chromoplasts, are fat soluble pigments. Before blooming stage, flowers have high chlorophyll content and show strong green color. At blooming stage, chlorophylls degrade while carotenoids increase and accumulate in chromoplasts. However, some genera of orchids, such as Cymbidium, Epidendrum, Cycnoches, and Catasetum are found to maintain chlorophylls (Arditti and Ernst, 1969). Dueker and Arditti (1968) showed that green Cymbidium flowers contains both chlorophyll a and b and can carry out photosynthesis. Carotenoids, producing yellow, orange, and red colors, are divided into three main groups: (i) highly oxidized xanthophylls, (ii) carotenes, and (iii) highly species-specific pigments (Goodwin, 1980).

Flavonoids, which are mostly water soluble pigments contained in vacuoles, produce colors in all visible spectra except green. Within this group, flavonols, chalcones, and aurones play important roles in giving yellow color (Robinson, 1975). Yellow flavonols owe their color to the presence of an extra hydroxyl (or methoxyl) group in the 6- or 8- position of the aromatic A-ring of their structures

(Harborne, 1982).

Yellow flowers usually contain xanthophylls such as zeaxanthin and its 5,8-epoxides, auroxanthin, and flavoxanthin, whereas deep orange flowers may have large amounts of β-carotene or alternatively lycopene (Vickery and Vickery, 1981; Harborne, 1982). Flavonoids make minor contributions to yellow color (Harborne, 1982).

Valadon and Mummery (1968a) stated that "the floral parts are yellow or orange depending on what carotenoids are present, which is the major one and the amount of total carotenoids, and also on the presence of other non-carotenoid pigments."

Goodwin (1980) compiled references about carotenoid distribution in flower petals from more than one hundred species. Some of them have a few carotenoids but some have up to 21 as in Rosa spp. This infers that carotenoid composition of many flower petals is complex (Spurgeon and Porter, 1980).

In Orchidaceae, over 100 flowers from different orchids were analysed by spectroscopic and chromatographic methods. The number of separated pigment compounds ranged from 6 to 25 in any given flower (Harper, 1972a). α-Carotene, β-carotene, lycoxanthin, xanthophyll, violaxanthin, and fucoxanthin were found in both the chromoplasts and chloroplasts of the flower tissue (Arditti and Ernst,

1969). In cattleya flowers, flavanones and flavanonols produce very light yellow or light cream color; flavones and flavonols, light yellow; and chalcones, aurones, and isoflavones, bright yellow (Harper, 1972a). Lowry and Keong (1973) found carotenoids in some Malaysian orchids, such as Spathoglottis aurea, Dendrobium crocatum, and Oncidium sphacelatum. Slc. Falcon'Alexanderi', a striking orange and red flower, contains a combination of both carotenoids and anthocyanins, whereas most of the bright yellow flowers, such as Blc. Jane Helton, contains mostly carotenoids (Harper, 1972a).

In Compositae, epoxy-carotenes and xanthophylls were found in large amounts and were the main pigments (Valadon and Mummery, 1967). In Rosaceae, epoxy carotenoids, including monoepoxy-, diepoxy- of α - and β-carotene and the epoxides of cryptoxanthin, lutein, and zeaxanthin were found in large amounts (Valadon and Mummery, 1968b). Over 80% of the carotenoids in Mimulus cypreus (V. Red Emperor) was β-carotene and the balance was small amounts of three xanthophylls. In contrast, about 1% of the total carotenoids in M. trigrinus was β-carotene and the remainder was a complex mixture of xanthophylls (Goodwin and Thomas, 1964). In Delonix regia of the family Leguminosae, which produces clusters of orange-red flowers, 34.0% by weight of the total carotenoids was β-carotene followed by 11.2%

γ-carotene, 10.0% rubixanthin, 7.7% phytoene, 5.0% cis-rubixanthin, 4.6% prolycopene, and 4.3% lycopene (Jungalwala and Cama, 1962). In Rhododendron species and cultivars, β-carotene, prolycopene, α-carotene-5,6-epoxide, lutein, and lutein-5,6-epoxide were the major carotenoid pigments found in yellow petals (Santamour and Dumuth, 1978).

2.2 Biosynthesis of Flower Pigments

2.2.1 Carotenoids

Biosynthesis of carotenoids has been reviewed recently by Spurgeon and Porter (1983) and Porter et al. (1984) The pathways start from the formation of isopentenyl pyrophosphate, followed by the formation of phytoene, lycopene, cyclic carotenes, and oxygenated carotenoids.

Two molecules of acetyl-CoA form isopentenyl pyrophosphate, a five-carbon compound, by six enzyme-catalyzed reactions. Intermediate compounds are acetoacetyl-CoA, HMG-CoA, mevalonic acid, mevalonic acid 5-phosphate, and mevalonic 5-pyrophosphate, respectively.

Isopentenyl pyrophosphate is isomerized to dimethylallyl pyrophosphate. It then condenses with a molecule of isopentenyl pyrophosphate to form geranyl pyrophosphate (C-10). Subsequently a molecule of

isopentenyl pyrophosphate is added to form farnesyl pyrophosphate (C-15) and geranylgeranyl pyrophosphate (C-20), respectively. Two molecules of geranylgeranyl pyrophosphate are converted to prephytoene pyrophosphate followed by cis-phytoene (C-40) in most organisms (Porter et al., 1984).

Phytoene is desaturated to phytofluene, ζ-carotene, neurosporene, and lycopene, respectively.

Lycopene is converted to cyclic carotenes, but little is known about the number and characteristics of the enzymes (Porter et al., 1984). The first cyclization reaction forms γ- and δ-carotenes and the second forms β- and α-carotenes.

After cyclization, xanthophylls, such as lutein and zeaxanthin occur by the addition of hydroxyl groups (Spurgeon and Porter, 1983). The pathway of lutein biosynthesis is not clearly known, but lutein probably is synthesized from α-carotene by the way of α-cryptoxanthin (Spurgeon and Porter, 1983). McDermott et al. (1974) studied alternative pathways of zeaxanthin biosynthesis in a Flavobacterium species by using nicotine as an inhibitor. They found that lycopene was converted into β-carotene under anaerobic conditions and into zeaxanthin in the presence of O_2. The alternative pathway of zeaxanthin biosynthesis was the conversion of rubixanthin into zeaxanthin via

β-cryptoxanthin.

In the violaxanthin cycle, occurring in the chloroplasts of higher plants, violaxanthin is formed by the epoxidation of zeaxanthin in which antheraxanthin occurs as an intermediate.

Carotenoid biosynthesis is regulated by environmental factors such as light, availability of oxygen, temperature, nutrition, and pH (Spurgeon and Porter, 1983). Photoregulation of carotenoid biosynthesis has been studied extensively by many workers.

2.2.2 Chlorophylls

Castelfranco and Beale (1983) reviewed recent advances and areas of current interest in chlorophyll biosynthesis. They reported that the earliest precursor of chlorophyll biosynthesis is δ-aminolevulinic acid (ALA), formed by the intact carbon skeleton of glutamate or α-ketoglutarate in plants and algae. Two ALA units form porphobillinogen (PBG) units. Four PBG units are then linked together in a head-to-tail sequence to produce an unstable linear tetrapyrrole followed by the formation of uroporphyrinogen III, coproporphyrinogen III, protoporphyrinogen IX, protoporphyrin IX, Mg-protoporphyrin IX, Mg-protoporphyrin IX monomethyl ester, Mg-2,4-divinyl-pheoporphyrin a_5, protochlorophyllide, chlorophyllide, geranylgeranyl

chlorophyllide, and chlorophyll a, respectively.

In the leaves of higher plants, chloroplasts normally develop from proplastids when the leaves are illuminated by visible light (Tevini, 1977). Etiolated leaves and etioplasts contain mainly protochlorophyllide, which will be converted by light to chlorophyllide.

According to Castelfranco and Beale (1983), "The final step of Chl a formation involves addition of the long chain polyisoprene phytyl moiety. This process is initiated by esterification of the propionic acid on ring D with geranylgeraniol (activated as the pyrophosphate ester) and subsequent reduction of the geranylgeranyl group to phytyl. The available evidence suggests that Chl b is derived directly from Chl a by oxidation of the methyl group on ring B to a formyl group".

2.2.3 Flavonoids

Flavonoids are the largest groups of plant phenolics that have C_{15} skeleton (C_6-C_3-C_6). Currently, 225 anthocyanidins and anthocyanins, 68 flavones and flavonols, and 486 flavone and flavonol glycosides are known (Harborne and Mabry, 1982). Knowledge of chemical compound structures in the flavonoid biosynthesis pathway come from tracer studies, which started around 1957 (Harborne *et al.*, 1975). They derive from products of two pathways, shikimic acid and

acetate-malonate pathways. Basically ring A is formed by head-to-tail condensation of three 'acetate units', whereas ring B and the three carbons of the central ring are derived from cinnamic acid (Vickery and Vickery, 1981).

Koukol and Conn (1961) discovered phenylalanine ammonialyase (PAL), the first enzyme of the phenylpropanoid pathway. That was the beginning of enzymic studies on flavonoids. Subsequently cell suspension cultures from various plants, such as soybean and parsley, were used for studying enzymic control in the biosynthesis of flavonoids. Nearly all of the enzymes involving in flavonoid biosynthesis have been purified from parsley cell cultures (Hahlbrock and Grisebach, 1979; Hahlbrock, 1981).

The role of PAL, which has been extensively studied, is to catalyze L-phenylalanine and L-tyrosine into trans-cinnamic acid + ammonia and trans-p-coumaric acid + ammonia, respectively. PAL activities are stimulated and increased by adding sucrose solution, light + CO_2, or red light resulting in increasing phenolic compound synthesis (Cam and Towers, 1977).

The addition reaction of p-coumaric and malonyl CoA, derived from acetic acid, produces a chalcone derivative. This is the key reaction in flavonoid biosynthesis, since chalcones are considered as the precursors of all other classes of flavonoid, such as aurones, flavanones, flavones,

flavonols, anthocyanins, isoflavonoids, etc. (Vickery and Vickery, 1981).

Pecket and Small (1980) investigated anthocyanoplasts, the intensely pigmented organelles found in anthocyanin-producing cells of more than 70 species, representing at least 33 families of angiosperms. Hypodermis and epidermis of hypocotyl and cotyledons of red cabbage (Brassica oleracea) were observed. Most rapid anthocyanin synthesis was found 2 days after germination. Also numerous unassociated small red vesicles were present in the vacuoles. By the following day the red vesicles appeared to associate and were fewer in number. Later one vesicle became substantially larger than the others. The larger red body observed 2-5 days of age increased in size from 3.2 μ - 10.6 μ and 2.4 μ - 8.1 μ in light and dark grown seedling cells, respectively. This large red body was identified as an anthocyanoplast. By using isolated protoplasts and vacuoles, they suggested that the anthocyanoplast was membrane-bounded, located in the cell vacuole, and was the site of anthocyanin biosynthesis. When pigment has been formed, it will leak into the cell vacuole.

Flavonoid biosynthesis in plants is regulated by various endogenous and exogenous factors. Most research has emphasized physiological rather than biochemical aspects. Some physiological factors, such as light and ionizing

radiation, temperature effects, water and atmospheric changes, carbohydrates and flavonoid levels, mineral nutrition, mechanical damage and pathogenic attack, and plant growth substances, were reviewed in detail by McClure (1975).

2.3 Function of Flower Pigments

Flavonoids produce all colors of the visible spectrum except green. Anthocyanins give purple, blue, orange, and red; flavones, flavonols, chalcones, and aurones produce yellow; flavanones and flavanonols are colorless, or slightly yellow (Robinson, 1975). They attract insects, birds, and animals, the agents of pollination and seed dispersal. Most correlations between flower color and the types of insects that visit them can be attributed, at least in part, to the flavonoids (Harborne *et al.*, 1975). Most of the species of Gramineae do not synthesize anthocyanins, presumably because their flowers are wind pollinated (Vickery and Vickery, 1981).

Pijl and Dodson (1969) listed some orchid species and their known pollinators in 113 genera. The major groups of pollinators that visit orchid flowers are bees, moths and butterflies, birds, and flies. Bees perceive ultraviolet light, but not red, so they react to blue, violet, purple, yellow, and white. Moths mostly fly at night, so flowers

tend to open at night and some close during the day. Flowers pollinated by moths are commonly white, creamy, or green. Butterflies fly in the daytime and prefer vivid colors including red. Birds tend to pollinate flowers with vivid colors, often scarlet or with contrasting parrot-like colors. Flies visit flowers of which color is often dull or green.

Harborne (1982) pointed out that the mixtures of two unrelated classes of yellow pigment, especially of carotenoids and yellow flavonoids, are often found in petals of members of the Compositae. This seems wasteful in terms of biosynthetic potential for plants to produce two classes of compounds to carry out the same function. In Rudbeckia hirta (Compositae), however there is separation of function of the two types of yellow pigment (Thompson et al., 1972; Harborne, 1982). The carotenoid provides the general yellow color in order to attract bees from a distance. Three flavonol glucosides, which show intense spectral absorption at 340 to 380 nm, are restricted in distribution to the petal bases. The flavonols act as a UV honey guide, directing the UV-sensitive bee once it has landed on the flower head to the nectar in the center of the blossom.

Flavonoids have other functions. They provide protection against damage from UV light because of their UV light absorption. Other functions include growth

regulation, enzyme inhibition, precursors of toxic substances, phytoalexins, and maintaining ion balance (Harborne et al., 1975; Harborne, 1977; Hahlbrock, 1981).

Carotenoids are found both in the chloroplasts of photosynthetic tissue (leaves and green tissue) and in the chromoplast of non-photosynthetic tissue (flowers, fruits, and other yellow and orange tissue). Carotenoids function as (i) accessory light-harvesting pigments in photosystem I and II for transfering radiant energy to chlorophyll (Cogdell, 1978), (ii) protective agents against photodynamic effects to chlorophylls (Krinsky, 1978), and (iii) the role of violaxanthin cycle, which has been extensively studied by many workers (Yamamoto et al., 1962; Sapozhnikov, 1973; Siefermann and Yamamoto, 1975). The role of violaxanthin cycle, which is not clearly known, is proposed by Sapozhnikov (1973) as oxygen evolution in photosynthesis. Siefermann and Yamamoto (1975) discussed oxygen evolution, NADPH oxidation function. These functions are not possible because of low oxygen uptake and no light requirement for epoxidation in isolated chloroplasts. In addition, they suggested that its function could be part of a regulatory system for photosynthesis, which functions at the membrane level by altering its properties due to the effects of violaxanthin/zeaxanthin ratio.

2.4 Degradation of Flower Pigments

It is accepted that phenolics are not the end products that accumulate unchanged in plant cells. There is continual synthesis, turnover, and degradation (Bell and Charlwood, 1980). Turnover is more rapid in floral tissues than in leaf tissues or other organs because the time requirement for floral pigmentation is quite short. Jones and Buchmann (1974) found morphological and physiological changes in floral color patterns following pollination in *Trigona fuscipennis* and *Trigona pectorallis*, which appeared to inhibit visitation by bees. In *Petunia hybrida*, the half life of anthocyanins based on delphinidin is only 25-31 hours (Steiner, 1971). In *Dendrobium* species and hybrids, flower longevity varies from 1-55 days, but different plants in one species or hybrid usually have the same longevity (Goh *et al.*, 1982).

Turnover of phenolic plant products may involve three types of reaction: (i) interconversion, (ii) catabolism, and (iii) oxidative polymerisation reactions (Barz and Hoesel, 1978). Removal of sugars from phenol glycosides is generally considered to be one of the first steps in catabolic pathways by glycohydrolases (Barz and Hoesel, 1978; Vickery and Vickery, 1981). Barz and Hoesel (1978) summarized various degradation experiments with labelled flavonoids in different cell suspension cultures and with

enzymes (mostly peroxidases) from various sources.

Color changes with age in petals has been studied in many plants. Most studies involved the relationships among anthocyanins, co-pigments (mostly flavone and flavonol), and the pH of epidermal cells. These studies were done with morning glory (Matile and Winkenbach, 1971; Asen et al., 1977), Fuchsia hybrida (Yazaki, 1976), and more than 250 plants of many families (Stewart et al., 1975). The results indicated that pH changed with age, resulting in changing of colors (Jurd, 1972; Stewart et al., 1975).

Yazaki (1976) studied co-pigmentation and color change with age in petals of Fuchsia hybrida. The blue-violet color of young Fuchsia petals appeared at pH 4.8 in the 1:0.6 molar ratio of anthocyanin to co-pigments. The color change from blue-violet in young petals to purple-red in old petals was caused by co-pigmentation and pH change from 4.8 to 4.2. The decrease of pH in the old petals was due to the increase of organic acids, such as aspartic, malic, and tartaric acids.

There is no uniform trend in color change of aging petals due to anthocyanins. Pigments remain stable in some flowers, decline drastically in others, whereas in some flowers a dramatic synthesis of anthocyanins is evident (Thimann, 1980). For example, at senescence, anthocyanins decrease sharply in chrysanthemums (Stickland, 1972) and

Lathyrus hirsutus (Pecket, 1966), but flavonol glycosides (quercetin and kaempferol) in Lathyrus hirsutus remain unchanged. In Digitalis purpurea, cv. Foxy, the loss of anthocyanin with aging is very small, and the corollas abscise without visible wilting or fading (Stead and Moore, 1977).

For carotenoids, oxidative processes take place with age, resulting in increasing concentration of oxygenated carotenoids as shown in Strelitzia and rose flowers (Valadon and Mummery, 1969; Thimann, 1980). In chrysanthemums, carotenoid and chlorophyll declined continuously from the bud stage whereas anthocyanin concentration reached a maximum in the half-open flower, and then decreased sharply (Stickland, 1972).

The change of color after pollination in orchids was first reported in 1899 (Anonymous) in Vanda coerulea, Odontoglossum X Andersonianum hebraicum, and Phalaenopsis lueddemanniana. Subsequently, research has been conducted on use of auxin and ethylene to induce change of color and senescence in orchid flowers (Akamine, 1963; Burg and Dijkman, 1967; Arditti et al., 1973; Chadwick et al., 1980; Strauss and Arditti, 1982). Arditti (1969) reviewed post pollination phenomena in orchid flowers. He concluded that in most flowers, petals and sepals will wilt, dry, and abscise. In Cattleya, sepals, petals, and labella will be

lost, but the column will change to green and remain on the flower. In Phalaenopsis, sepals and petals will change to green and conduct photosynthesis to yield energy for the development of the seed pod. In Vanda, pollination will cause flower color to fade in less than 20 hours, similar to the effect of auxin treatment.

2.5 Color Inheritance

2.5.1 Flavonoids

A number of genetical studies on flower color of ornamental crops, such as Antirrhinum majus, Cheiranthus Cheiri, Dahlia variabilis, Dianthus caryophyllus, Lathyrus odoratus, Pelagonium zonale, Petunia hybrida, Primula sinensis, Streptocarpus spp., and Verbena spp. were reviewed by Scott-Moncrieff (1936) and Harborne (1976). In addition, color inheritance in tissues of plant, excluding flower tissue, was also studied extensively. Studies included root color in Brassica spp. (Hoshi, 1975; Hoshi and Hosoda, 1978) and Raphanus spp. (Hoshi et al., 1963), and the color of aleurone tissue of Zea mays (Reddy and Coe, 1962; Reddy and Reddy, 1975; Reddy and Peterson, 1977 and 1978).

According to the flavonoid biosynthetic pathway, the inheritance of anthocyanins, flavones, and flavonols are related to one another because they are derived from the

same precursor. All genes associated with the synthesis of each compound can block one another. If the genes that control the beginning of the pathway are homozygous recessive, they will not produce the compound and will block the pathway to prevent the synthesis of other compounds which occur at the later steps. Anthocyanin production (blue to red) requires more dominant genes than flavones and flavonols (white or pale yellow) (Harper, 1974; Kho *et al.*, 1977).

In *Antirrhinum majus*, three color factors P, M, and Y are proposed with respect to the aglycones of flavone, flavonol, aurone, and anthocyanin glycosides (Geissman *et al.*, 1954; Jorgensen and Geissman, 1955a and 1955b). P factor controls the oxidation of the C_3-fragment that joins the two six-carbon ring of the flavonoid. M factor controls the oxidation of the B-ring. Y factor, which operates at an earlier stage than P and M factors, controls only the amount of aurone pigment.

In *Petunia hybrida*, white flowering mutants accumulate dihydroflavonol intermediate due to genetic blocks in the biosynthetic pathway leading to anthocyanins (Kho and Bennink, 1975; Kho *et al.*, 1977). The gene An1, converting flavonols into anthocyanins, is an unstable gene. The mutation of gene An1 changes the dark red-flowered cultivar 'Roter Vogel' into the white flowered plants, and the back

mutations will cause red spots on the white flowers (Bianchi *et al.*, 1978; Mulder *et al.*, 1981)

In *Brassica* spp., Hoshi (1975) found that two pairs of genes controlled red, purple, and white colors of the root. This result was also found in *Raphanus* spp. (Hoshi *et al.*, 1963). It was shown that in amphidiploid species of *Brassica*, the flavonoid components came out together as a sum of flavonoids appearing in both diploid ancestors. Moreover, there was some correlation between genome and flavonoid pattern (Hoshi and Hosoda, 1978).

In *Zea mays*, extensive work has been done on the genetic control of flavonoid synthesis in the aleurone tissue of maize (Coe, 1978). The genes C, C2, R, A, A2, Bz, Bz2, and Pr are required for the formation of purple color, and the recessive genes control non-purple colors (red, bronze, and colorless) (Reddy and Coe, 1962; Reddy and Reddy, 1975; Reddy and Peterson, 1977). The recessive gene *in* increases the quantity of anthocyanins without changing any qualitative differences in the pigment composition (Reddy and Peterson, 1978)

2.5.2 *Carotenoids*

The genetic control of carotenoid biosynthesis has been studied extensively in tomato fruits (*Lycopersicon esculentum*) and in carrot roots (*Daucus carota*) (Goodwin,

1980). Fruit colors (Thompson, 1961; Zscheile and Lesley, 1967; Sink et al., 1974), the pathway of carotenoid biosynthesis (Kargl et al., 1960), and the nutritive value as provitamin A (Lincoln and Porter, 1950; Premachandra et al., 1976) are the main objectives in genetic studies of carotenoids in tomato fruits. For carrots, genetic studies of carotenoids in root colors were reported by Imam and Gableman (1968) and Laferriere and Gableman (1968).

In tomato fruits, about 90-95% of total carotenes are lycopene and about 5% are β-carotene, which is provitamin A (Lincoln and Porter, 1950; Thompson, 1961). Lincoln and Porter (1950) and Premachandra et al. (1976) made crosses by using varieties with high β-carotene and lycopene content as the parents to get higher provitamin A content than the normal varieties. Lincoln and Porter (1950) found that intermediate β-carotene amount was found in the F_1 generation. In the F_2 generation, β-carotene was found at the levels 0-24.9%, 25-74.9%, and 75-100% in the ratio of 1:2:1. Therefore, they assumed that β-carotene and lycopene differed by a single incompletely dominant gene.

Laferriere and Gabelman (1968) studied five color phenotypes of carrot, namely, white, yellow, orange tinge, intermediate orange, and orange. White was dominant to all other colors and completely inhibited the synthesis of α- and β-carotene in the F_1 progenies.

Imam and Gabelman (1968) studied three color phenotypes in carrot root: orange (high total carotenoids and high α- and β-carotene), light orange (low total carotenoids and high amount of xanthophylls), and lemon (very low in total carotenoids, high xanthophylls and a small fraction of β-carotene). A monogenic difference was found. Light orange was dominant over orange, and lemon was dominant over light orange.

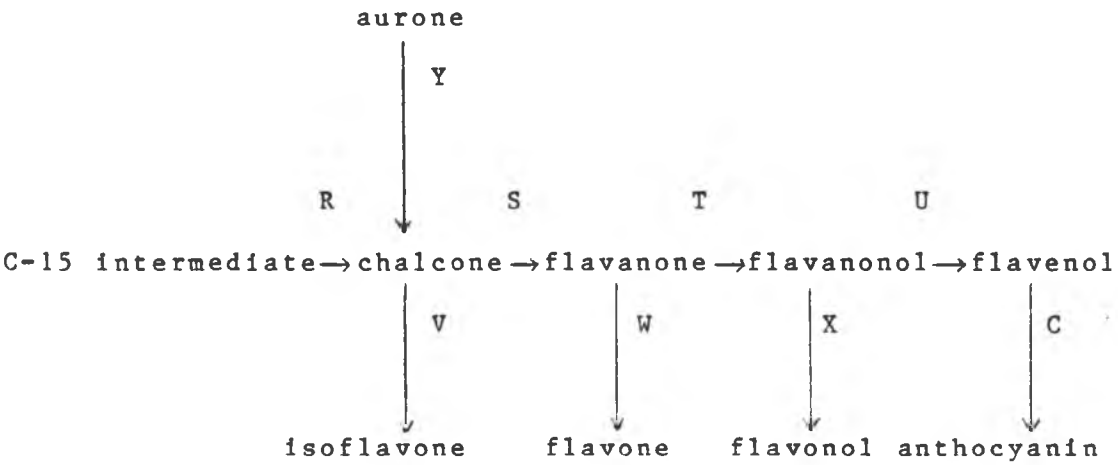
2.6 Color Inheritance in Orchids

Genetics of flower color in orchids was first reported by Hurst (1925). He found that two complementary factors called C and R were involved in color inheritance. The R gene is an uncolored chemical precursor of color (chromogen) and the C gene controls the production of an enzyme to convert colorless chromogen into colored pigments. For colored flowers, C and R may be present in a homozygous state (-CC and RR-) or in a heterozygous state (-Cc and Rr-), and albinos will have either cc or rr, or both.

Storey and Kamemoto (1960) studied inheritance of semi-alba (flowers having white sepals and petals with colored lips) in a cattleya pedigree. They made selfing from a hybrid, C. Rubio (dark lavender flowers) X C. warscewiczii 'Firmin Lambean' (white flowers). The offsprings were 41 colored : 8 semi-alba : 17 white

(9:3:4). The genotype of this hybrid was determined to be CCRrPp based on the ratio of offsprings and its pedigree. When gene p is homozygous recessive (pp), it will suppress the color of sepals and petals. Therefore, semi-alba will have the genotype C-R-pp.

Harper (1974) proposed a scheme of genetic control of flavonoids basicly on flavonoid biosynthesis pathway in which each letter represented a gene that controlled the step as follows:



In the genus Dendrobium, the results of the dendrobium breeding program at the University of Hawaii indicated that lavender and purple were dominant to white. Yellow-flowered color of hybrids faded if both parents did not have bright yellow flowers. Color purity was improved in D. Jaquelyn Thomas 'Y 166-1' by selection and inbreeding (Bobisud and

Kamemoto, 1982).

Many yellow dendrobium cultivars are polyploids and their backgrounds come from section Ceratobium and section Phalaenanthe (Kamemoto, 1980). Both sections are closely related as indicated by the high percentage of successful intersectional crosses (Wilfret and Kamemoto, 1969). Many species in the section Ceratobium have bright yellow flowers, but flowers are often small, such as those of D. undulatum, D. gouldii, and D. schulleri. Species in the section Phalaenanthe have large and attractive white, lavender, or purple flowers, such as those D. phalaenopsis.

In subtribe Oncidiinae, yellow flowers are common. In section Variegata, yellow color is dominant to red color, masking the reds or confining the red color to the back of the flowers. If the yellow is not strong, red and yellow show equally. Color is determined by what occurs in the lip and tepal colors do not have much influence. Therefore, the color on the back of the lip is an important consideration in further breeding (Moir and Moir, 1980 and 1982).

In the cattleya alliance, Cattleya dowiana var. aurea is a yellow that is recessive to lavender and purple (Fenton, 1951; Bennett, 1965). Crossing with Cattleya gigas (purple) results in all purple in the F_1 generation and 3:1 ratio of purple : yellow in F_2 generation (Woodward, 1965). The yellow of Laelia flava is dominant over the

purple of cattleyas (Fenton, 1951; Sweet *et al.*, 1960; Bennett, 1965; Woodward, 1965; Marlowe, 1981). *Cattleya aurantica* is dominant in the production of yellow hybrids (Woodward, 1965; Dangler, 1980). Red hybrids have *Sophronitis orandiflora* in their backgrounds (Bennett, 1965; Woodward, 1965). *Brassavola digbyana* gives light soft shades of green color and a large fringed lip (Bennett, 1965; Woodward, 1965). *Cattleya bicolor*, *Cattleya forbesii*, *Cattleya granulosa*, *Cattleya guttata*, *Cattleya velutina*, and *Epidendrum mariae* impart their green color to their hybrids (Woodward, 1965).

The superb pink *Phalaenopsis* of today trace their ancestry back to two species, *Phalaenopsis schillerana* and *Phalaenopsis sanderana*. Breeding for yellow *Phalaenopsis* has been slow because there was little foundation for good yellow color until recent years. Usually the yellow colors fade or may be hidden in the first generation and will show in the following generation (Freed, 1979).

Griesbach (1984) studied carotenoid-anthocyanin combinations on flower color. Novel flower colors can be created by combining carotenoid and anthocyanin pigments. For example, the hybrid between *Phalaenopsis amboinensis* (yellow with brown spot) X *Doritaenopsis* Grebe (margenta) produces flowers that are bronze. He suggested that breeding for improved flower color should be concerned with

anthocyanin/carotenoid mixtures, pH, copigmentation, and individual pigments (anthocyanins, carotenoids, or chlorophylls).

Harper (1972b) presented his concepts of color inheritance in orchids as follows:

"1. Structure genes and regulator genes operate together to control color in orchid flowers, with up to several dozen genes being involved in the production of color in a given orchid.

2. The concentration of various flavonoids derived from a common precursor and the concentration of various carotenoids derived from a common precursor appear to be controlled independently.

3. Carotenoids and flavonoids are derived from different precursors and are genetically independent.

4. The chemical compounds present in the lips of orchids are generally different from those present in the other flower parts, indicating gene differences between the lip and the petals and sepals.

5. Different cells in the same flower part may differentiate to produce different pigments or different concentrations of the same pigment, thus leading to spots and various shades of color. The epidermal cells on the front and back of a petal for instance may produce different compounds."

III. MATERIALS AND METHODS

3.1 Carotenoid and Chlorophyll Identification in Flowers of Dendrobium Species and Hybrids

Dendrobium species and hybrids used in this study were part of the University of Hawaii *Dendrobium* breeding program. Fifteen species (Table 1) representing 6 sections, as classified by Schlechter (1912), and 23 cultivars and selections (Table 2) were analyzed for carotenoids and chlorophylls in flower petals. All the species except *D. phalaenopsis* (*Phalaenanthe*) were yellow-flowered. All hybrids involved species from sections *Ceratobium* and *Phalaenanthe*. Some of the cultivars had received awards from both the American Orchid Society (AOS) and the Honolulu Orchid Society (HOS).

In this study, flower color was described visually and matched with the color chart of the Royal Horticultural Society, London.

3.1.1 Extraction of Pigments

An inflorescence with blooming flowers was harvested and immersed in tap water for 15 min. Petals, 2-4 gm fresh wt depending on availability and color intensity, were measured accurately and homogenized in 100 ml acetone. The slurry was filtered through glass wool using a Buchner

Table 1. Petal color of *Dendrobium* species used for carotenoid and chlorophyll determination in their petals.

Plant no.	Plant name	Petal color	Author of species name
Section	*Callista*		
-	*D. aggregatum*	bright yellow	W. Roxburgh
Section	*Ceratobium*		
K741-23	*D. antennatum*	yellow-green	J. Lindley
D284	*D. conanthum*	yellow-green	R. Schlechter
D153	*D. gouldii*	yellow-green	H. Reichenbach
D174	*D. helix*	bright yellow	-
D99-1	*D. ionoglossum*	yellow-green	R. Schlechter
K321	*D. schulleri*	yellow-green	J. J. Smith
D200-1	*D. stratiotes*	yellow-green	H. Reichenbach
K737	*D. strebloceras*	greyed-orange	H. Reichenbach
D36-2	*D. undulatum*	greyed-orange	R. Brown
D43-1	*D. undulatum*	greyed-orange	R. Brown
D270	*D. undulatum* var. *broomfieldii* 'Shimonishi'	yellow-green	Fitzgerald
Section	*Eleutheroglossum*		
D173-2	*D. canaliculatum*	yellow-green	R. Brown
Section	*Eugenanthe*		
-	*D. moschatum*	yellow-orange	O. Swartz
Section	*Latourea*		
-	*D. macrophyllum*	pale yellow	A. Richard
67381	*D. spectabile*	pale yellow	F. Miquel
Section	*Phalaenanthe*		
D37-2	*D. phalaenopsis*	white	Fitzgerald
TR-2	*D. phalaenopsis*	purple	Fitzgerald

Table 2. Petal color of *Dendrobium* cultivars and selections used for carotenoid and chlorophyll determination in their petals.

Plant no.	Plant name	Petal color
D269	*D.* Alice Queen (*D.* Alice Chong X *D.* Bronze Queen)	yellow-green
D211-1	*D.* Amy 'Orange' (*D.* *undulatum* X *D.* Hula Girl)	greyed-orange
D227	*D.* Betty Ho 'Kamiya' (*D.* Misty Green X *D.* Maynouk)	greyed-yellow
D186-1	*D.* Betty Ho 'Waimea' (*D.* Misty Green X *D.* Maynouk)	yellow
D242	*D.* Caesar (*D.* *phalaenopsis* X *D.* *stratiotes*)	pale yellow
D237-2	*D.* Cathy X *D.* Aina Haina X *D.* Gold Coast X *D.* Liholiho	yellow-green
D234-2	*D.* C. K. Ai. 'Oka' (*D.* May Neal X *D.* Hula Girl)	yellow-bronze
D217-1	*D.* Esther Zane Shigaki 'Butterfly' (*D.* Edythe Pung X *D.* Fiftieth State Beauty)	yellow-green
D167	*D.* Field King (*D.* Liholiho X *D.* May Neal)	yellow-bronze
D245	*D.* Field King AM/AOS (*D.* Liholiho X *D.* May Neal)	yellow-green
D216-1	*D.* Imelda Romualdez (*D.* Milroy X *D.* Fiftieth State Beauty)	yellow-bronze
D244	*D.* Mary Mak (*D.* Theodore Takiguchi X *D.* May Neal)	yellow-green
D231	*D.* Mary Trowse (*D.* Hula Girl X *D.* *schulleri*)	yellow-green
D179B	*D.* May Neal 'Srisobhon' (*D.* Hawaii X *D.* *schulleri*)	bright yellow
D180	*D.* May Neal X *D.* *schulleri*	yellow-green
D232-19	*D.* Ng Eng Cheow (*D.* Alice Spalding X *D.* Jaquelyn Thomas)	yellow-green
D265-B	*D.* Pakanoa 'Waianae Beauty' (*D.* Manoa X *D.* Pakanu)	yellow-green
D254-1	*D.* Prince Kuhio X *D.* Liholiho	yellow-green

Table 2. Contd.

Plant no.	Plant name	Petal color
H19-1	*D.* Salak (*D. stratiotes* X *D. undulatum*)	yellow-bronze
D190	*D.* Ukio (*D.* Neo-Hawaii X *D. stratiotes*)	yellow-green
K432	*D. strebloceras* X *D. canaliculatum*	greyed-orange
K528	*D.* Caesar X *D.* May Neal 'Srisobhon'	pale yellow
K528	from bud (2-3.2 cm)	yellow
K528	from 2-4 wks after blooming	white
K637	*D.* Jaquelyn Thomas X *D.* May Neal 'Srisobhon'	yellow-purple
K637	from bud (2-3.2 cm)	yellow-purple
K637	from 2-4 wks after blooming	white-purple
K650	*D.* May Neal 'Srisobhon' X *D. helix*	bright yellow
K751	*D.* Caesar X *D. canaliculatum*	pale yellow

funnel. The residue was extracted repeatedly with more acetone until the filtrate was colorless. The pigments were washed with petroleum ether by adding an equal volume of petroleum ether to the acetone extract in a separatory funnel. All of pigments were transferred to the upper ether layer. The ether layer was collected, concentrated to dryness in a rotary evaporator at 35°C, and redissolved in 1 ml acetone. The pigments were subsequently stored at -20°C for use in high-performance liquid chromatography (HPLC). The extraction was conducted in the dark. Reagents used were of analytical grade.

3.1.2 HPLC System

The sample volume between 2-20 µl, depending on concentration, was injected into the HPLC (Beckman Model 334 Gradient System). A reversed-phase column, 10 um LiChrosorb RP 18 (250 mm length X 4.6 mm internal diameter, Alltech Associates) was installed, and gradient elution was set as described by Braumann and Grimme (1981). Reagent water, HPLC grade methanol, and analytical grade acetonitrile were used in the solvent system. Solvent A was water and solvent B was methanol-acetonitrile (25:75 V/V). The proportion of solvent B was increased in a linear gradient from 75% to 100% in 20 min, followed by maintaining 100% of solvent B until all pigments were eluted. The flow rate was 1.5

ml/min and a variable wavelength UV detector (Hewlett-Packard Model 1030B) was set at 445 nm.

3.1.3 Identification of Pigments

Major peaks from HPLC chromatograms were collected and their absorption spectra recorded with a UV-VIS spectrophotometer (Perkin-Elmer Model 554). The sample was then added with a drop of 0.05N ethanolic HCl (Jungalwala and Cama, 1962) to identify epoxy carotenoids by hypsochromic shift. Retention times and absorption maxima of sample were compared with those of the known compounds extracted from spinach leaves, in addition to absorption maximal comparison with data from Braumann and Grimme (1981).

3.1.4 Quantitative Determination

Each known compound was collected from HPLC, its solvent removed under vacuum evaporation at 35°C, and dissolved in ethanol or acetone (Table 3). The absorbance of each compound was recorded and its specific extinction coefficient at λmax (Table 3) was used to calculate the amount of the compound according to the formula by Davies (1976) as follows:

Table 3. Specific extinction coefficients at λ max of carotenoids and chlorophylls used for quantitative determination.

Pigment	$E^{1\%}_{1cm}$	λ (nm)	Solvent	Reference
Neoxanthin	2243	439	Ethanol	Davies (1976)
Violaxanthin	2550	443	Ethanol	Davies (1976)
Antheraxanthin	2350	446	Ethanol	Davies (1976)
Lutein	2550	445	Ethanol	Davies (1976)
Zeaxanthin	2340	452	Acetone	Davies (1976)
Chlorophyll b	535	647	Acetone	Vernon (1960)
Chlorophyll a	926	663	Acetone	Vernon (1960)
β-Carotene	2620	453	Ethanol	Davies (1976)

$$X = \frac{Ey}{E^{1\%}_{1cm} \times 100}$$

where:

X is amount of a carotenoid, gm,

E is the extinction at the given wavelength,

y is volume of solution, ml,

$E^{1\%}_{1cm}$ is specific extinction coefficient.

Known amounts of each compound were then injected into the HPLC system and the peak height was used as a criterion in comparison for quantitative determination.

3.2 Degradation of Flower Pigments in Dendrobium Hybrids

Progenies of three crosses (K528, K637, and K650), made at the University of Hawaii were examined for degradation of flower pigments.

K528 (pale yellow petals) is a hybrid from D. Caesar (pale yellow) X D. May Neal 'Srisobhon' (yellow).

K637 (white-purple petals) is a hybrid from D. Jaquelyn Thomas (white-purple) X D. May Neal 'Srisobhon' (yellow).

K650 (yellow petals) is a hybrid from D. May Neal 'Srisobhon' (yellow) X D. helix (yellow).

3.2.1 Pigment Changes at Different Stages of Flowers in a Single Raceme

Different stages of flowers in a single raceme from three crosses were numbered from the flower that had just opened as stage 1, followed down toward the raceme base to stage 10, respectively. The absorption spectra of flavonoids, carotenoids, and chlorophylls were measured with a Unicam Sp 1800 Ultraviolet spectrophotometer.

Flavonols, anthocyanins, and chlorophylls were extracted by immersing 250 mg fresh wt of petals in 5 ml of 0.1% hydrochloric acid in methanol under refrigeration (about 9°C) for 48 hrs. Anthocyanin and chlorophyll content were recorded directly from the extract by using absorbance at 530 and 654 nm, respectively (Stickland, 1972). For flavonols, the extract was diluted 10 times and the absorbance was recorded at 260 nm (Pecket, 1966).

Carotenoids were extracted by immersing 250 mg fresh wt of petals in 5 ml of 20% petroleum ether in methanol under refrigeration (about 9°C) for 48 hrs (Stickland, 1972). The absorbance was recorded at 470 nm.

3.2.2 Carotenoid and Chlorophyll Changes in Growth and Development of Flowers

Methods of qualitative and quantitive identification of carotenoids and chlorophylls were described in section 3.1.

In K528 and K637, 3 stages of flowers were examined: 1. -bud stage (2.0-3.2 cm bud length), 2. -young stage

(blooming), 3. -old stage (2-4 wks after blooming).

3.3 Cytology and Yellow Flower Color Inheritance

3.3.1 Cytology

The chromosome numbers of yellow-bronze Dendrobium accessions were determined from root smears. The excised root-tips were pretreated in 0.002M hydroxyquinoline at 16°C for about 4 hrs, fixed in 1:1:2 mixture of 95% ethanol, chloroform, and glacial acetic acid for 10 min at 16°C, hydrolyzed in 1N hydrochloric acid for 3 min at 60°C, and stained in 1% aceto-orcein (Wilfret and Kamemoto, 1971).

3.3.2 Yellow Flower Color Inheritance

Progenies of some crosses and their parents (Table 4) were observed for inheritance of flower pigments. These progenies had yellow to yellow-bronze flowers or had parents with yellow to yellow-bronze flowers.

Variation of flower color was examined from progenies of some available crosses, which had yellow flowered ancestors. Flower color was observed visually and compared with the Colour Chart of the Royal Horticultural Society. Carotenoids and chlorophylls were determined by using the method described in section 3.1.

Table 4. Petal color of *Dendrobium* plants used for yellow flower color inheritance.

Plant no.	Plant name	Petal color
D173-2	*D. canaliculatum*	yellow-green
D242	*D.* Caesar (*D. phalaenopsis* X *D. stratiotes*)	pale yellow
D167	D. Field King (*D.* Liholiho X *D.* May Neal)	yellow-bronze
D174	*D. helix*	bright yellow
D179B	*D.* May Neal 'Srisobhon' (*D.* Hawaii X *D. schulleri*)	bright yellow
K737	*D. strebloceras*	greyed-orange
D193	*D.* Spellbound (*D.* Valley King X *D.* Pakanu)	white
K44-50	*D.* Jaquelyn Thomas 'Uniwai Blush' (*D. phalaenopsis* 'Lyon's Light No.1' X *D. gouldii*)	white with pink tinge
K159-21	*D.* Jaquelyn Thomas (K44-50 selfed)	white with pink tinge
K250	K44-50 X *D.* Field King	variation
K382	K159-21 X *D.* Field King	variation
K432	*D. strebloceras* X *D. canaliculatum*	greyed-orange
K487	K250-29 X *D.* May Neal 'Srisobhon'	variation
K500	K250-29 selfed	variation
K526	K250-29 X *D.* Field King	variation
K528	*D.* Caesar X *D.* May Neal 'Srisobhon'	pale yellow
K642	*D.* Spellbound X K382-18	variation
K650	*D.* May Neal 'Sri Sobhon' X *D. helix*	bright yellow
K662	K159-21 X K487-131	white
K751	*D.* Caesar X *D. canaliculatum*	pale yellow

IV. RESULTS AND DISCUSSION

4.1 Carotenoid and Chlorophyll Identification in Flowers of Dendrobium Species and Hybrids

Carotenoids and chlorophylls from petals of the genus Dendrobium were identified by comparing their retention times, absorption maxima, and hypsochromic shifts with those of the standards prepared from spinach leaf extract. Absorption maxima reported by Braumann and Grimme (1981) were also compared to confirm the results. The eight peaks from HPLC chromatogram were identified as neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b and a, and β-carotene, (Table 5).

The absorption spectra of eight identified pigments in solution of methanol/acetonitrile (25:75 V/V) are shown in Figures 1-8. Zeaxanthin (Figure 5) and β-carotene (Figure 8) showed a shoulder at the shorter wavelength of the absorption maxima. After adding a small drop of concentrated hydrochloric acid, a hypsochromic shift of about 20 nm for mono-epoxides was found in neoxanthin (Figure 1) and antheraxanthin (Figure 3), and about 40 nm for di-epoxides in violaxanthin (Figure 2).

Replications were not run because the machines are known to be highly accurate, it takes about 2 hours to run each sample, and, in many cases, there was only 1 plant

Table 5. Identification of carotenoids and chlorophylls from petals of the genus Dendrobium.

Peak no.	Dendrobium RT[Z]	Dendrobium AM[Y]	Dendrobium HS[X]	Spinach RT	Spinach AM	Spinach HS	Braumann and Grimme (1981) AM	Identification
1	19.5	414, 438, 467	20	19.5	412, 438, 466	20	414, 438, 467	Neoxanthin
2	21.9	418, 442, 467	40	21.9	418, 440, 468	40	418, 441, 471	Violaxanthin
3	24.7	422, 445, 472	20	24.7	416, 445, 472	20	422, 445, 472	Antheraxanthin
4	26.8	422, 445, 473	--	26.8	420, 444, 472	--	422, 447, 475	Lutein
5	27.4	426, 450, 478	--	27.4	426, 450, 478	--	---	Zeaxanthin
6	28.2	460, 645	--	28.2	460, 646	--	460, 648	Chlorophyll b
7	32.0	426, 659	--	32.0	424, 661	--	431, 664	Chlorophyll a
8	40.2	424, 450, 476	--	40.2	424, 450, 476	--	426, 453, 479	β-Carotene

[Z]RT = retention time (min).
[Y]AM = absorption maxima (nm) in methanol/acetonitrile (25:75 V/V).
[X]HS = hypsochromic shift (nm).

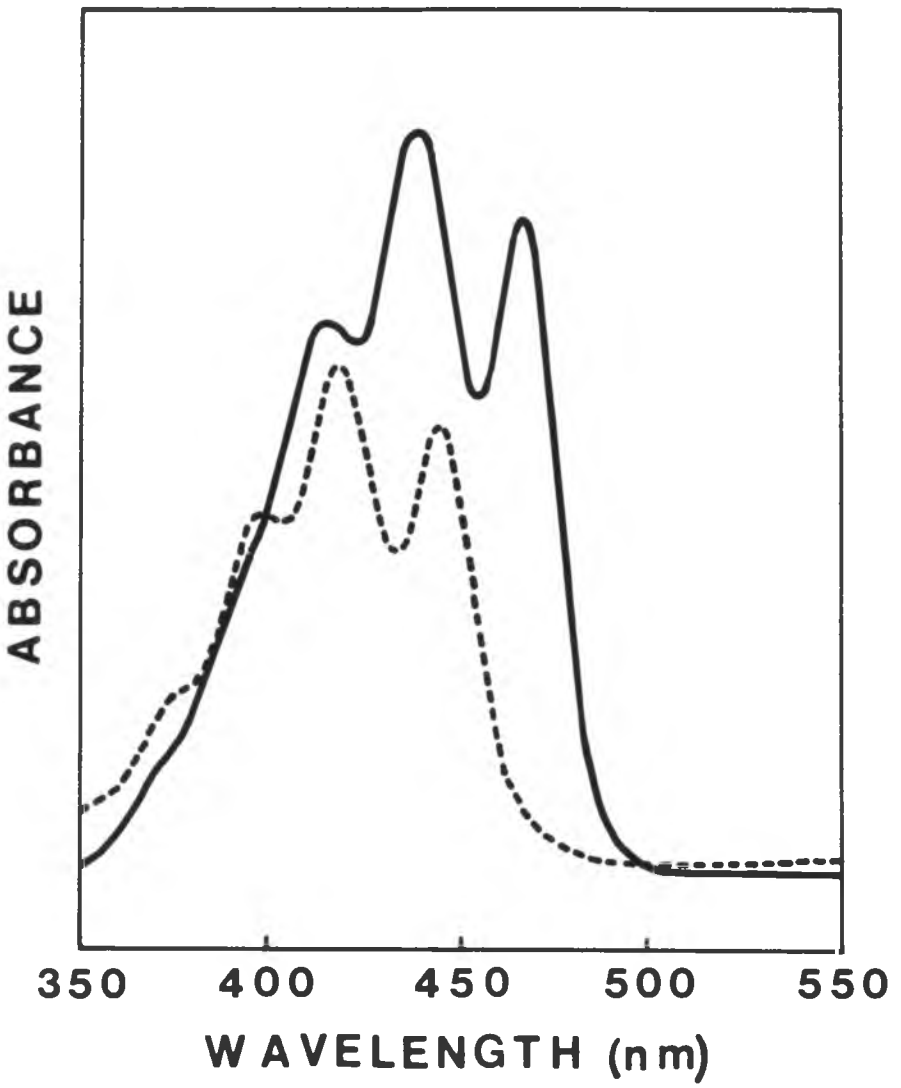


Figure 1. Absorption spectra of neoxanthin (Peak no. 1) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (- - -).

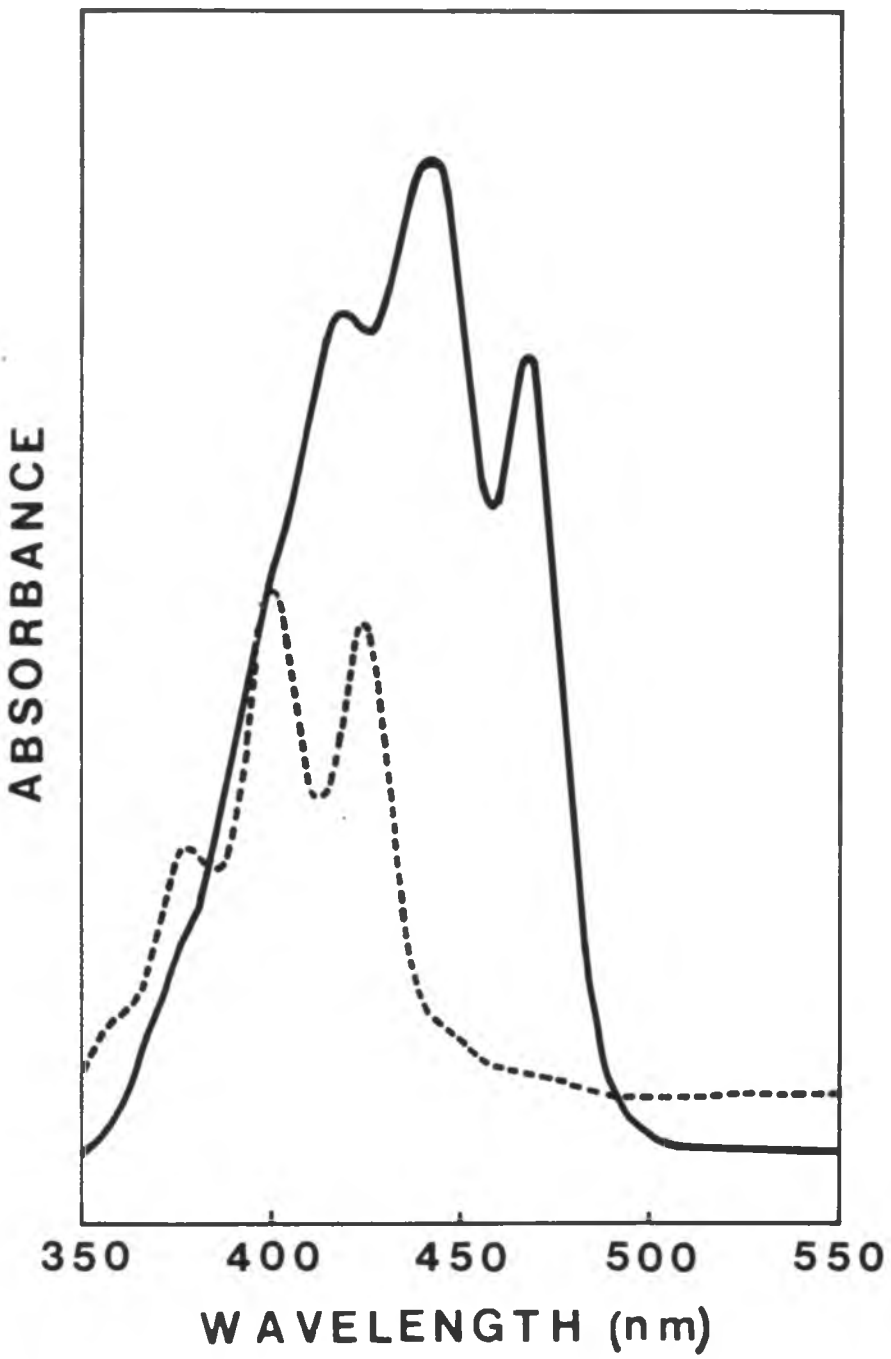


Figure 2. Absorption spectra of violaxanthin (Peak no. 2) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (---).

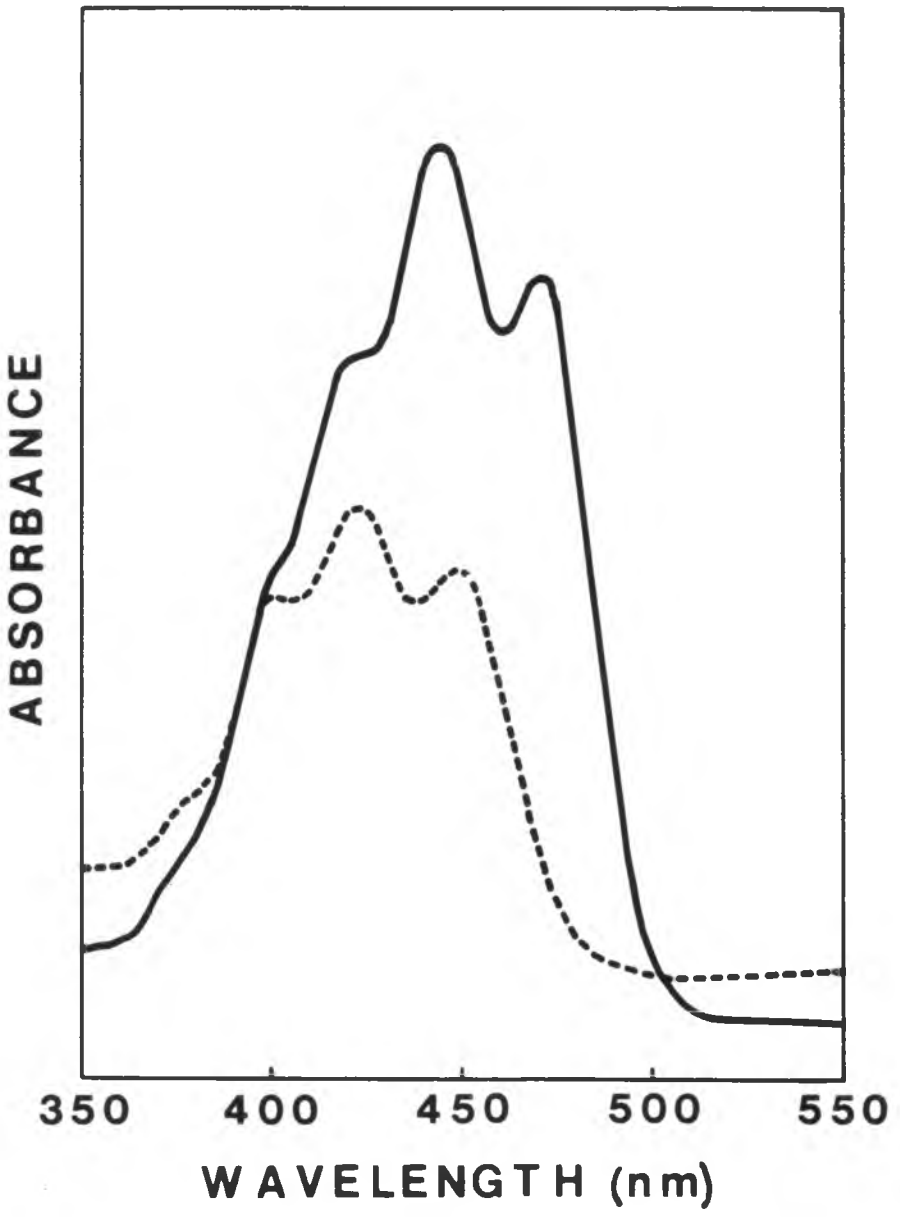


Figure 3. Absorption spectra of antheraxanthin (Peak no. 3) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (- - -).

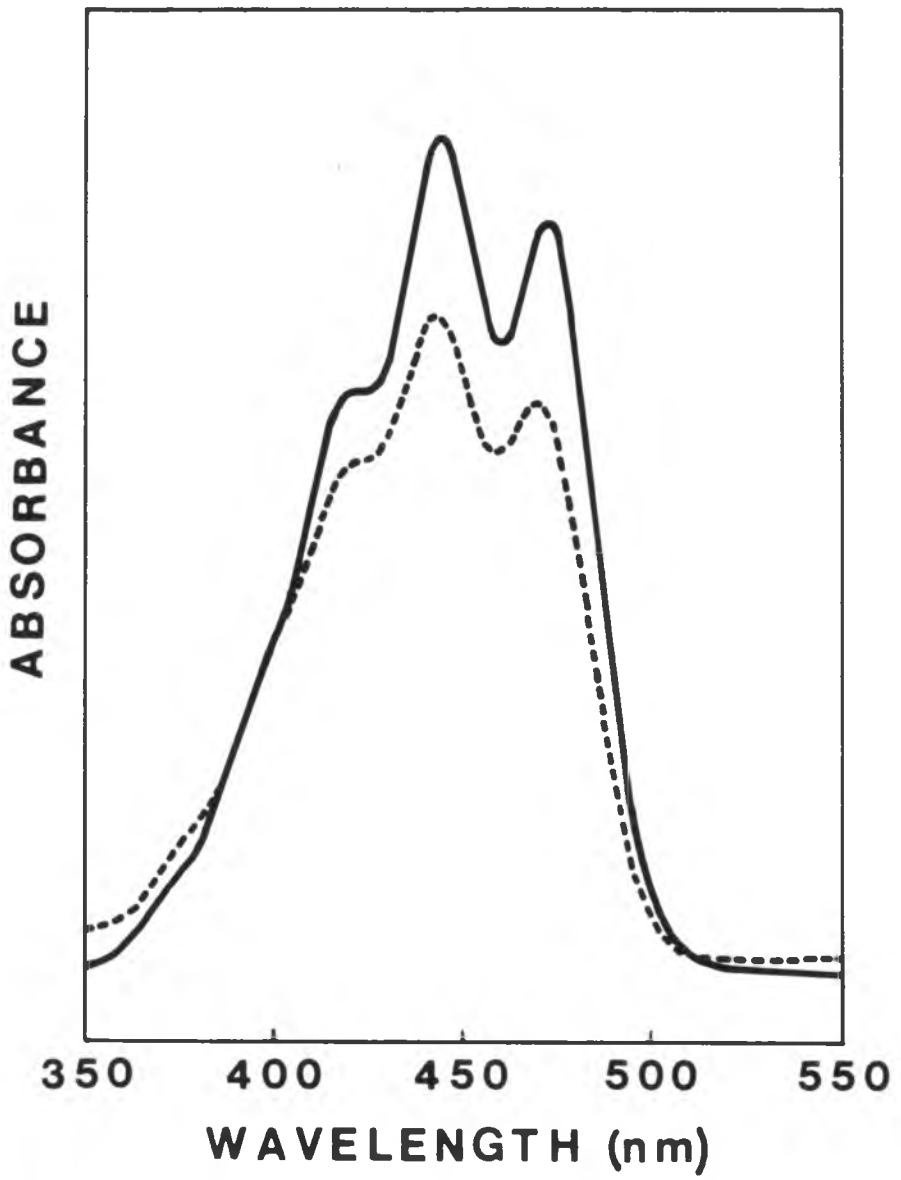


Figure 4. Absorption spectra of lutein (Peak no. 4) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (---).

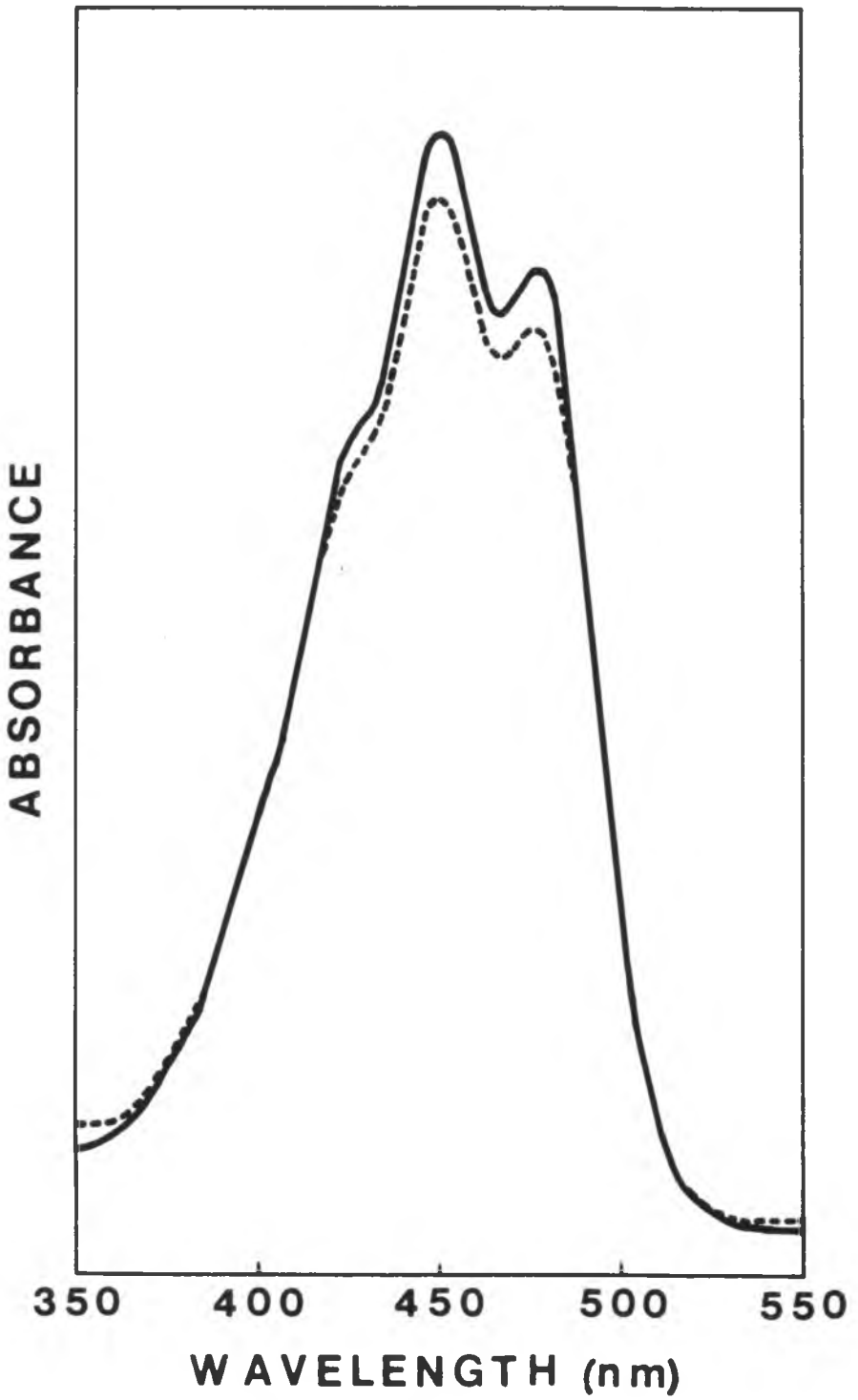


Figure 5. Absorption spectra of zeaxanthin (Peak no. 5) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (- - -).

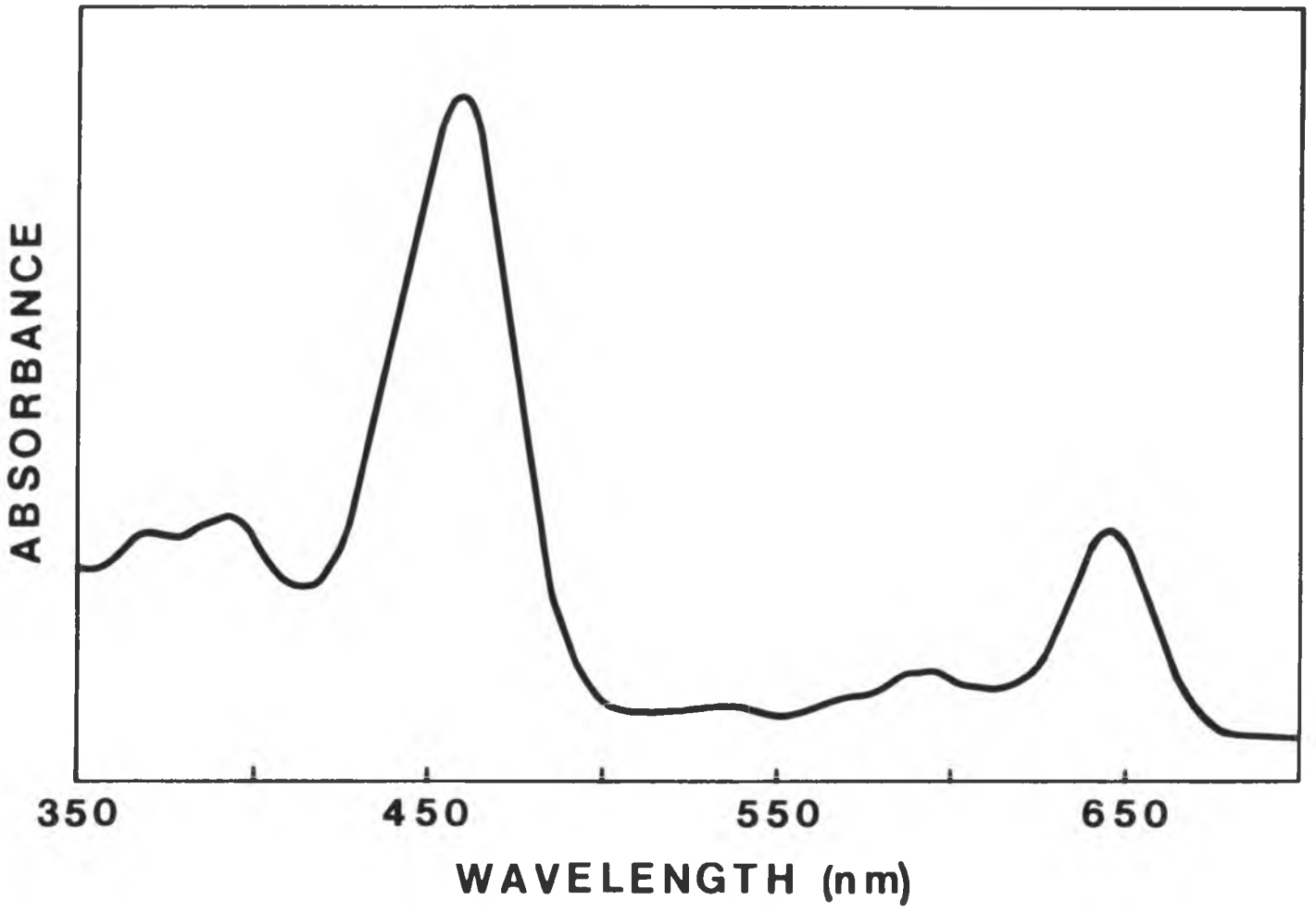


Figure 6. Absorption spectrum of chlorophyll b (Peak no. 6) in methanol/acetonitrile (25:75 V/V).

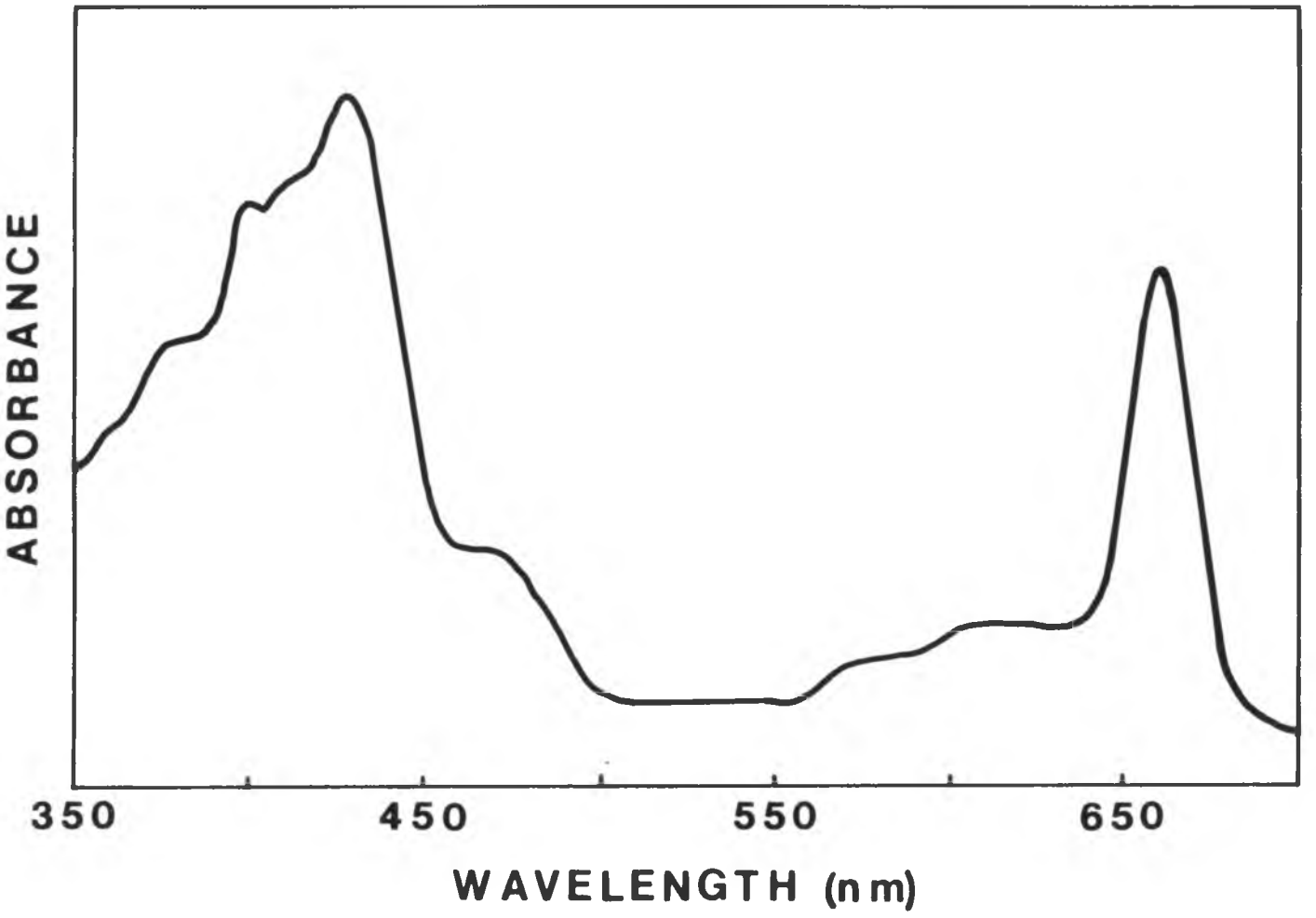


Figure 7. Absorption spectrum of chlorophyll a (Peak no. 7) in methanol/acetonitrile (25:75 V/V).

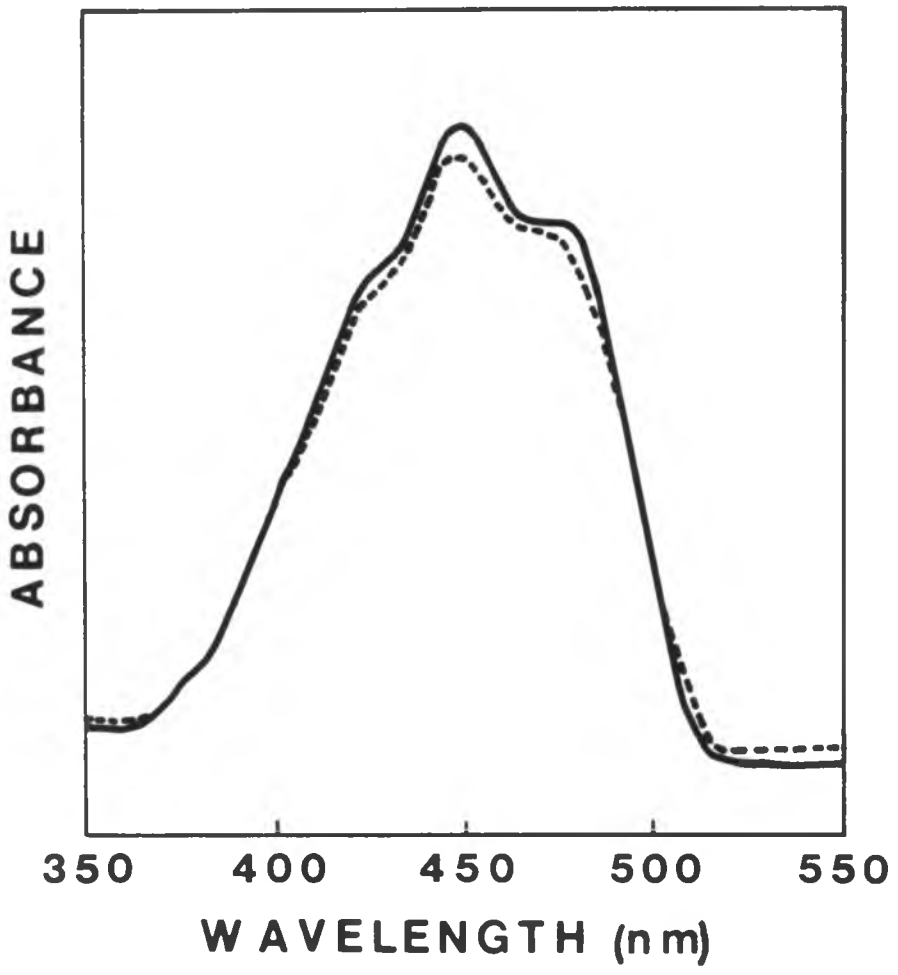


Figure 8. Absorption spectra of β-carotene (Peak no. 8) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (---).

available which bloomed only once a year. The accuracy of the determinations is shown by the two values for D. macrophyllum (Table 6). Although the total amounts of pigments vary greatly between the two dates, the percentages are very similar.

In section Callista, only D. aggregatum was examined. This species had pure bright yellow petals (Figure 9). Neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin were detected. Zeaxanthin was found in the highest percentage followed by antheraxanthin, lutein, neoxanthin, and violaxanthin (Table 7; Figure 10).

In section Ceratobium, 9 species were examined. Most of these species had color variation, and flower color was a mixture of green and some other colors. D. helix had yellow flower color (Figure 11). Eight major compounds were detected with lutein and neoxanthin in highest percentages (Figure 12). D. antennatum, D. conanthum, D. gouldii, D. ionoglossum, D. schulleri, D. stratiotes, and D. undulatum var. broomfieldii had shades of yellow-green flower color, ranging from 150A to 152C. They had high percentages of lutein and chlorophyll a and b. D. undulatum (D36-2 and D43-1) and D. strebloceras (Figure 13) had greyed-orange flowers in which pigments were a mixture of carotenoids, chlorophylls, and anthocyanins. Eight major compounds were detected in both D. undulatum accessions, but only lutein,

Table 6. Quantitative distribution of carotenoids and chlorophylls in petals of D. macrophyllum at two different time.

Time	Carotenoids (%)						Total (µg/g fresh wt)	Chlorophylls (%)		Total (µg/g fresh wt)
	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-Carotene		Chl. a	Chl. b	
Feb. 6, 1984	4.0	3.2	3.6	8.2	1.6	9.7	30.3	13.2	4.7	17.9
	(13.2)[Z]	(10.6)	(11.9)	(27.0)	(5.3)	(32.0)		(73.7)	(26.3)	
Mar. 8, 1984	1.7	1.3	1.5	3.9	0.7	4.2	13.3	5.6	2.1	7.7
	(12.8)	(9.8)	(11.3)	(29.3)	(5.2)	(31.6)		(72.7)	(27.3)	

[Z]numbers in parenthesis is the percentage of that compound.

Table 7. Quantitative distribution of carotenoids and chlorophylls in petals of some Dendrobium species.

Plant no.	Name	Color R H S[Z]	Carotenoids (%) Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-Carotene	Total (μg/g fresh wt)	Chlorophylls (%) Chl. a	Chl. b	Total (μg/g fresh wt)
Section Callista												
-	D. aggregatum	13B[Y]	5.3	3.1	25.8	22.7	43.1	-	35.7	-	-	-
Section Ceratobium												
K741-23	D. antennatum	150B	9.5	8.7	3.2	61.1	0.8	16.7	25.2	65.9	34.1	40.2
D284	D. conanthum	151A	10.4	5.9	3.0	35.1	3.2	42.4	129.9	56.6	43.4	100.8
D153	D. gouldii	152C	7.2	0.9	3.2	42.3	3.4	43.0	44.4	54.3	45.7	62.6
D174	D. helix	7B	19.8	8.8	7.5	50.7	5.8	7.4	63.7	53.2	46.8	49.1
D99-1	D. lonoglossum	152C	14.7	9.8	5.3	48.9	4.4	16.9	86.8	60.0	40.0	104.6
K321	D. schulleri	153C	9.9	6.2	3.7	27.1	4.0	49.1	270.7	63.0	37.0	164.8
D200-1	D. stratiotes	154C	7.9	8.9	12.9	26.8	6.9	36.6	10.1	54.0	46.0	12.6
K737	D. strebloceras[X]	168B	-	-	-	100.0	-	-	53.4	86.5	13.5	178.6
D36-2	D. undulatum	164B	10.6	4.1	4.1	33.1	3.6	44.5	153.3	77.2	22.8	74.1
D43-1	D. undulatum	174A	10.4	5.4	7.3	46.0	4.9	26.0	70.0	68.6	31.4	47.8
D270	D. undulatum var. broomfieldii 'Shimonishi'	151A	10.6	6.4	7.4	55.2	-	20.4	108.1	61.0	39.0	62.3
Section Eleutheroglossum												
D173-2	D. canaliculatum	154B	6.3	7.2	3.6	16.3	1.7	64.9	162.1	67.4	32.6	127.9
Section Eugenanthe												
-	D. moschatum	21C	2.9	4.4	12.4	16.9	63.3	-	83.2	-	-	-
Section Latourea												
-	D. macrophyllum	8C	12.8	9.8	11.3	29.3	5.2	31.6	13.3	72.7	27.3	7.7
67381	D. spectabile	3D	12.1	5.3	3.4	38.1	3.9	37.2	20.7	39.6	60.4	5.3
Section Phalaenanthe												
D37-2	D. phalaenopsis	155D	-	-	-	-	-	-	-	-	-	-
TR-2	D. phalaenopsis	78B	-	-	-	-	-	-	-	-	-	-

[Z]R H S = Colour Chart from The Royal Horticultural Society, London.

[Y]No. 3-13 = yellow, 21 = yellow-orange, 78 = purple, 150-154 = yellow-green, 155 = white, 164-174 = greyed-orange.

[X]D. strebloceras has some unknown carotenoids which have in high concentrations.

Figure 9. Flowers of D. aggregatum.

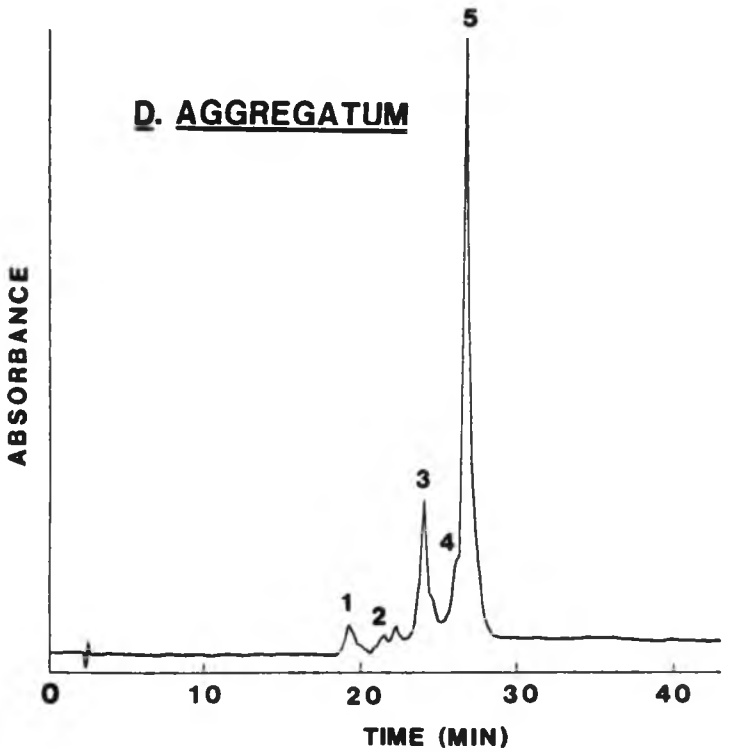


Figure 10. HPLC chromatogram of a total pigment extract of D. aggregatum. Peak 1 = neoxanthin, Peak 2 = violaxanthin, Peak 3 = antheraxanthin, Peak 4 = lutein, Peak 5 = zeaxanthin.

Figure 11. Flowers of D. helix.

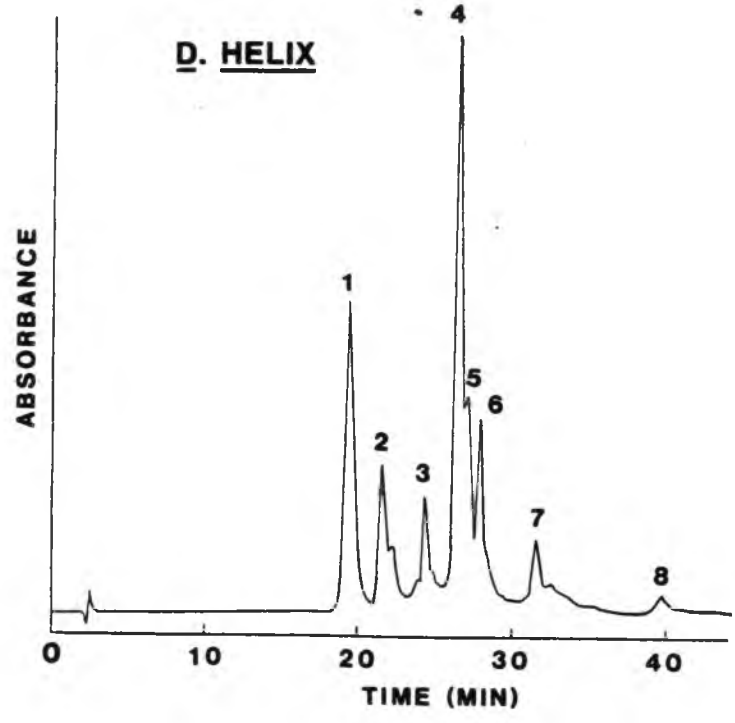


Figure 12. HPLC chromatogram of a total pigment extract of D. helix. Peak 1 = neoxanthin, Peak 2 = violaxanthin, Peak 3 = antheraxanthin, Peak 4 = lutein, Peak 5 = zeaxanthin, Peak 6 = chlorophyll b, Peak 7 = chlorophyll a, Peak 8 = β-carotene.

Figure 13. Flowers of D. strebloceras.

chlorophyll a and b, and some unknown pigments were detected in D. strebloceras (Figure 14).

The unknown pigments in D. strebloceras, peaks A and B (Figure 14), were carotenoids because they each had three absorption maxima (Appendix Figures 30 and 31). Peak A showed absorption maxima at 428, 454, and 485 nm, while peak B showed absorption maxima at 427, 451, and 480 nm with a shoulder at the shorter wavelength. Since neither absorption spectrum showed a hypsochromic shift, the compounds were not epoxy carotenoids. They were probably carotenes because their retention times were close to β-carotene.

In general in this section, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin were found in small amounts whereas lutein was found in large amounts, especially in the yellow to yellow-bronze flowers of D. schulleri, D. undulatum (D36-2), and D. undulatum var. broomfieldii 'Shimonishi'. Chlorophyll a was detected in a higher amount than chlorophyll b.

In section Eleutheroglossum, only D. canaliculatum was available. The petals of this species were yellow-green at the proximal portion and white at the axial portion. All eight major compounds were detected (Table 7). β-carotene, chlorophyll a and chlorophyll b were found in high proportions.

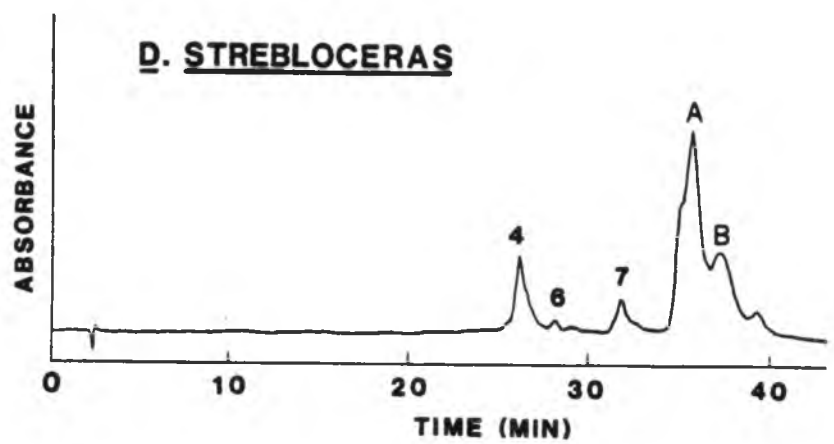


Figure 14. HPLC chromatogram of a total pigment extract of D. strebloceras. Peak 4 = lutein, Peak 6 = chlorophyll b, Peak 7 = chlorophyll a, Peaks A and B = unknown carotenoids.

In section Eugenanthe, only D. moschatum was examined. Its flowers were pure bright yellow-orange (Figure 15). Neoxanthin, violaxanthin, antheraxanthin, lutein, and zeaxanthin were found (Table 7). However, β-carotene and chlorophylls a and b were not detected. Zeaxanthin was found in the highest proportion (Figure 16).

Two species in section Latourea, D. macrophyllum and D. spectabile, were examined. All eight major compounds were detected, but in small amounts because the petals were pale yellow in color (Table 7). Lutein and β-carotene were major compounds in the flowers.

In section Phalaenanthe, two plants of D. phalaenopsis were examined. One had white petals and the other had purple petals. No carotenoids or chlorophylls were detected in either plants (Table 7).

The pigment distribution in some Dendrobium hybrids is shown in Table 8. The hybrids had flower colors ranging from yellow to red-purple. Flowers of all the hybrids contained 8 major compounds except D. Amy 'Orange' in which chlorophyll a and b were not detected, and flowers of D. Caesar in which β-carotene was not detected.

The proportions of pigments in hybrids affected their flower color. For example, D. Amy 'Orange', with a greyed-orange flower color, contained β-carotene in the highest amount (Table 8). D. Ng Eng Cheow, with creamish

Figure 15. Flowers of D. moschatum.

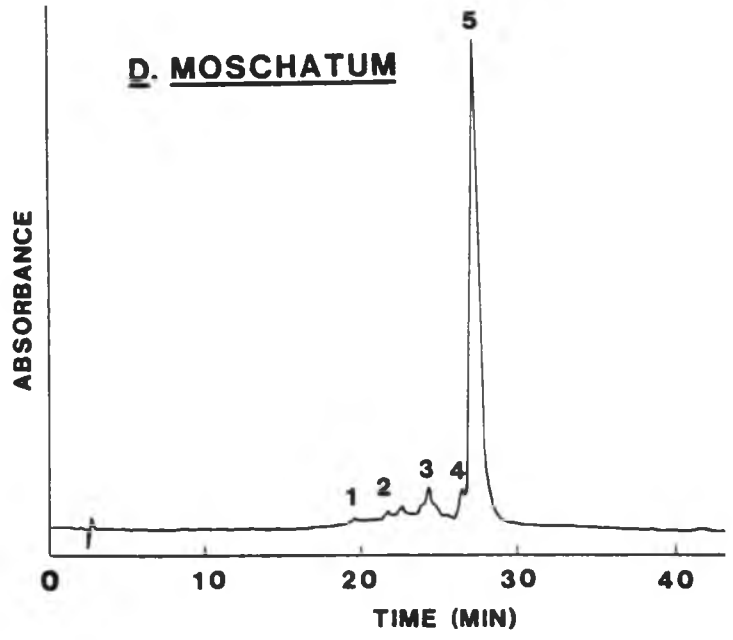


Figure 16. HPLC chromatogram of a total pigment extract of D. moschatum. Peak 1 = neoxanthin, Peak 2 = violaxanthin, Peak 3 = antheraxanthin, Peak 4 = lutein, Peak 5 = zeaxanthin.

Table 8. Quantitative distribution of carotenoids and chlorophylls in petals of some Dendrobium hybrids.

Plant no.	Name	Color R H S[Z]	Carotenoids (%) Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-Carotene	Total (µg/g fresh wt)	Chlorophylls (%) Chl. a	Chl. b	Total (µg/g fresh wt)
D269	D. Alice Queen	154B[Y]	4.0	2.9	2.4	28.4	3.1	59.2	119.0	54.5	45.5	102.5
D211-1	D. Amy 'Orange'	172B	11.7	4.1	6.0	20.7	4.9	52.6	119.6	-	-	-
D227	D. Betty Ho'Kamiya'	1C	12.1	11.4	6.3	28.6	4.0	37.6	63.0	58.1	41.9	51.6
D186-1	D. Betty Ho 'Waimea'	2D	11.1	9.5	7.9	28.9	3.6	39.0	30.5	45.5	54.5	24.4
D242	D. Caesar	155B	12.5	12.5	18.8	50.0	6.2	-	1.6	72.7	27.3	5.5
D237-2	D. Cathy X D. Aina Haina X D. Gold Coast X D. Lihollho	151A	14.7	8.5	4.1	39.7	3.9	29.1	61.2	64.9	35.1	76.1
D234-2	D. C. K. Al.'Oka'	183B+153D	10.7	5.8	3.9	19.8	16.9	42.9	99.2	52.2	47.8	163.1
D217-1	D. Esther Zane Shigaki 'Butterfly'	151C	10.2	11.4	5.2	20.1	3.8	49.3	42.2	62.3	37.7	51.7
D167	D. Field King	160A+166D	12.7	10.1	6.9	21.9	4.2	44.2	55.2	54.0	46.0	53.0
D245	D. Field King AM/AOS	154B	7.6	4.3	2.3	30.5	3.3	52.0	103.1	52.1	47.9	69.1
D216-1	D. Imelda Romualdez	183B+151C	16.7	8.2	4.3	37.0	2.0	31.8	213.3	51.6	48.4	101.9
D244	D. Mary Mak	154C	26.3	14.1	9.4	19.3	3.2	27.7	40.4	62.5	37.5	27.5
D231	D. Mary Trowse	151B	12.4	10.1	7.5	33.1	4.9	32.0	34.7	64.2	35.8	42.7
D179B	D. May Neal'Srisobhon'	7A	10.3	5.8	2.9	24.0	2.4	54.6	78.8	60.6	39.4	62.0
D180	D. May Neal X D.schulleri	150B	27.7	19.8	6.0	30.0	3.3	13.2	30.3	50.9	49.1	22.0
D232-19	D. Ng Eng Cheow	149D	9.9	16.8	6.8	30.4	8.4	27.7	19.1	64.1	35.9	56.9
D265-B	D. Pakanoa 'Walanae Beauty'	151A	18.7	8.3	2.8	41.7	3.0	25.5	185.3	62.3	37.7	170.8
D254-1	D. Prince Kuhio X D. Lihollho	151C	10.2	6.2	4.7	31.3	2.8	44.8	107.4	53.5	46.5	81.1
H19-1	D. Salak	153A+74C	12.0	10.9	10.5	26.9	4.6	35.1	45.8	67.8	32.2	75.9
D190	D. Ukio	154D	13.5	21.3	13.5	20.6	8.1	27.0	14.1	59.6	40.4	9.9

[Z]R H S = Colour Chart from The Royal Horticultural Society, London.

[Y]No. 1 = green-yellow, 2-7 = yellow, 74 = red-purple, 149-154 = yellow-green, 155 = white, 160 = greyed-yellow, 166-172 = greyed-orange, 183 = greyed-purple.

green flowers, contained chlorophyll a and b in highest amounts. D. May Neal X D. schulleri with pale yellow-green flowers had small amounts of all 8 compounds. D. Mary Mak has slightly darker yellow-green flowers than D. May Neal X D. schulleri which is reflected by similar distribution of carotenoids except a slightly higher concentration of β-carotene.

The bright yellow flower group, D. Alice Queen, D. Betty Ho 'Kamiya', D. C. K. Ai 'Oka', D. Field King (D245), D. Imelda Romualdez, D. May Neal 'Srisobhon', D. Pakanoa 'Waianae Beauty', and D. Prince Kuhio X D. Liholiho contained high concentrations of both lutein and β-carotene (Table 8). Neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin were in lower proportions.

Flower color in relation to pigment composition is difficult to interpret if the flowers have many colors involved. In many dendrobiums, three groups of pigments, flavonoids, carotenoids, and chlorophylls, are involved. These pigments do not only show their distinct colors, but also mix with one another; for example, greyed-orange results from a yellow, orange, and green mixture. Neoxanthin, violaxanthin, antheraxanthin, and lutein are yellow, but zeaxanthin and β-carotene are yellow with an orange shade. Chlorophylls a and b are green. Since all carotenoids have yellow colors, it is difficult to explain

which carotenoid plays an important role in flower color except by using the percentage of pigments as a criterion.

Table 9 shows the percentage of each carotenoid and chlorophyll, and the total amount of each group of pigments. In the yellow species group, lutein and β-carotene were the most prevalent pigments except in D. aggregatum, which had no β-carotene, but had high zeaxanthin and antheraxanthin. D. helix, with a higher intensity of yellow color, had the highest total amount of carotenoids. The total amount of chlorophylls in this group was not as high as in the yellow-green and greyed-orange groups. In the yellow-green group, lutein, β-carotene, and the chlorophylls were the major pigments. D. canaliculatum, D. conanthum and D. schulleri, with high intensity of color, had high total amounts of both carotenoids and chlorophylls. In the bright yellow-orange D. moschatum, zeaxanthin was 63.3% of the carotenoids. No chlorophylls were detected. In the greyed-orange D. undulatum accessions, lutein and β-carotene were found in the highest percentages.

4.1.1 Discussion

Table 5 shows the identification of carotenoids and chlorophylls which is based on retention time of the peak from HPLC, absorption maxima, and hypsochromic shifts from spectrophotometer. Retention time of 8 peaks from

Table 9. Percentage and total amounts of carotenoids and chlorophylls in relation to petal color of some yellowish-flowered *Dendrobium* species.

Petal color and species	Carotenoids (%)						Total (μg/g fresh wt)	Chlorophylls (%)		Total (μg/g fresh wt)
	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-Carotene		Chl. a	Chl. b	
Yellow										
D. aggregatum	5.3	3.1	25.8	22.7	43.1	-	35.7	-	-	-
D. helix	19.8	8.8	7.5	50.7	5.8	7.4	63.7	53.2	46.8	49.1
D. macrophyllum	12.8	9.8	11.3	29.3	5.2	31.6	13.3	72.7	27.3	7.7
D. spectabile	12.1	5.3	3.4	38.1	3.9	37.2	20.7	39.6	60.4	5.3
Yellow-green										
D. antennatum	9.5	8.7	3.2	61.1	0.8	16.7	25.2	65.9	34.1	40.2
D. canaliculatum	6.3	7.2	3.6	16.3	1.7	64.9	162.1	67.4	32.6	127.9
D. conanthum	10.4	5.9	3.0	35.1	3.2	42.4	129.9	56.6	43.4	100.8
D. gouldii	7.2	0.9	3.2	42.3	3.4	43.0	44.4	54.3	45.7	62.6
D. lonoglossum	14.7	9.8	5.3	48.9	4.4	16.9	86.8	60.0	40.0	104.6
D. schulleri	9.9	6.2	3.7	27.1	4.0	49.1	270.7	63.0	37.0	164.8
D. stratiotes	7.9	8.9	12.9	26.8	6.9	36.6	10.1	54.0	46.0	12.6
D. undulatum var. *broomfieldii* 'Shimonishi'	10.6	6.4	7.4	55.2	-	20.4	108.1	61.0	39.0	62.3
Yellow-orange										
D. moschatum	2.9	4.4	12.4	16.9	63.3	-	83.2	-	-	-
Greyed-orange										
D. undulatum(D36-2)	10.6	4.1	4.1	33.1	3.6	44.5	153.3	77.2	22.8	74.1
D. undulatum(D43-1)	10.4	5.4	7.3	46.0	4.9	26.0	70.0	68.6	31.4	47.8

Dendrobium petal extract and spinach leaf extract were the same. This inferred that each peak that has the same retention time may be a representative of the same compound. In addition, absorption maxima of pigments from Dendrobium petal and spinach leaf extract were similar. This confirmed that pigments from both extracts were the same. When absorption maxima were compared with data on pigments from spinach (Braumann and Grimme, 1981), the absorption maxima were not as close as from our own spinach leaf extract. This may be due to the differences in the instrumentation.

The procedure for carotenoid and chlorophyll identification takes about 1 hr for extraction and another hour for HPLC. It provides a more accurate and rapid analysis of pigments than paper or thin layer chromatography. Retention times of all compounds were longer than the data from Braumann and Grimme (1981) and Ben-Amotz et al. (1982). For example, retention time for β-carotene in this experiment was about 40.2 min but its retention time reported by Braumann and Grimme (1981) was about 28 min. From HPLC chromatogram (Figures 10, 12, 14, and 16), all peaks were clearly separated. Braumann and Grimme (1981) were not able to separate zeaxanthin from lutein, but in the present study, good separation was obtained between both compounds if there were large amounts

of zeaxanthin; otherwise, zeaxanthin eluted as a shoulder behind the lutein peak. Ben-Amotz et al. (1982) also found good separation between zeaxanthin and lutein.

In Table 7, some relationships among sections were observed. Sections Ceratobium, Eleutheroglossum, and Latourea have all 6 carotenoids and chlorophyll a and b except D. strebloceras, from section Ceratobium. This species has only lutein, chlorophyll a and b, and some unknown pigments, which are carotenoids eluted between chlorophyll a and β-carotene. Section Latourea has small amounts of all 8 compounds, compared to the other two sections. Although only one species each in sections Callista and Eugenanthe was studied, it may be significant that β-carotene and chlorophyll a and b were not detected in D. aggregatum and D. moschatum. Both showed high concentrations of zeaxanthin, lutein, and antheraxanthin.

Wilfret and Kamemoto (1969) and Kamemoto and Wilfret (1980) studied crossability of species between sections in Dendrobium as well as the meiotic behavior of intersectional hybrids. They concluded that sections Ceratobium and Eleutheroglossum were closely related, more distantly related to Latourea, and most distantly related to Callista and Eugenanthe. Moreover, sections Callista and Eugenanthe were not closely related because of the difficulty in producing intersectional crosses. The relationships based

on presence or absence of carotenoids and chlorophylls in the present study generally agree with their conclusions. Although sections Callista and Eugenanthe showed similarity in pigment compositions (Table 7), it can not be concluded that both sections are closely related, because only one species from each section was observed. However, it suggests that biochemical studies may be useful in the taxonomy of Dendrobium.

The hybrids investigated are polyploids, and their ancestors include many generations of hybridization within the sections Ceratobium and Phalaenanthe (Kamemoto, 1980). Many species in the section Ceratobium have bright yellow flowers, but are small and often unattractive. D. phalaenopsis of the section Phalaenanthe has large and attractive white, lavender, or purple flowers. Pedigrees of some cultivars and selections, listed in Table 8, are shown in the Appendix (Figures 32-44). Most ancestors are species from section Ceratobium, such as D. gouldii, D. grantii, D. schulleri, D. stratiotes, D. taurinum, D. tokai, and D. undulatum; and from section Phalaenanthe, such as D. bigibbum and D. phalaenopsis. Carotenoids and chlorophylls were not detected in species from section Phalaenanthe (Table 7); therefore, all the hybrids (Table 8) have carotenoids and chlorophylls which were inherited from species in the section Ceratobium. If carotenoids and

chlorophylls from more species and species hybrids can be identified, then the inheritance of yellow color might be elucidated and would contribute to the improvement of yellow Dendrobium cultivars.

4.2 Degradation of Flower Pigments in Dendrobium Hybrids

4.2.1 Pigment Changes at Different Stages of Flowers in a single raceme

From observation in the saranhouse, young flowers of K528 (Figure 17) and K637 (Figure 19) had some green and yellow color near the center of the flowers, but both colors faded rapidly after blooming. Therefore, mature flowers of K528 and K637 appeared to be white with purple tinge and light purple, respectively. In contrast, flowers of K650 (Figure 21) were bright yellow and the yellow color persisted for more than a month.

The absorbance of anthocyanins, flavonols, carotenoids, and chlorophylls of petals of K528, K637, and K650 at different stages is shown in Table 10. The graphs of all pigment components in petals of the hybrids are shown in Figures 18, 20, and 22.

In K528 (Table 10; Figure 18), anthocyanins were found in low concentration throughout 10 stages. Carotenoids decreased rapidly from stages 1 to 3, and then remained

Table 10. Absorbance of flower pigments in three Dendrobium crosses at different stages of flowers in a single raceme.

Cross	Stage[Z]	Anthocyanins (530nm)	Flavonols (260nm)	Carotenoids (470nm)	Chlorophylls (654nm)
K528	1	0.013	11.700	0.245	0.055
	2	0.015	9.500	0.084	0.048
	3	0.005	7.550	0.052	0.032
	4	0.006	9.300	0.047	0.031
	5	0.017	7.900	0.045	0.030
	6	0.005	8.800	0.046	0.019
	7	0.002	8.800	0.030	0.014
	8	0.000	8.600	0.036	0.014
	9	0.000	8.800	0.034	0.012
	10	0.000	9.500	0.034	0.012
K637	1	0.285	8.600	0.245	0.078
	2	0.265	8.000	0.118	0.052
	3	0.265	8.200	0.091	0.046
	4	0.205	7.500	0.068	0.041
	5	0.255	7.650	0.066	0.039
	6	0.310	8.000	0.039	0.041
	7	0.240	7.700	0.035	0.030
	8	0.262	7.500	0.035	0.030
	9	0.225	7.500	0.019	0.028
	10	0.193	7.100	0.032	0.023
K650	1	0.046	11.800	1.130	0.178
	2	0.040	8.750	1.240	0.150
	3	0.038	8.700	0.985	0.154
	4	0.041	10.400	1.080	0.152
	5	0.038	8.450	1.100	0.138
	6	0.040	7.850	1.100	0.141
	7	0.043	9.000	1.080	0.152
	8	0.039	8.500	1.220	0.134
	9	0.047	9.550	1.245	0.161
	10	0.038	9.150	1.070	0.133

[Z]Stage is numbered from the flower that has just opened as stage 1, followed down toward the raceme base to stage 10, respectively.

Figure 17. Flowers of K528 and its parents.

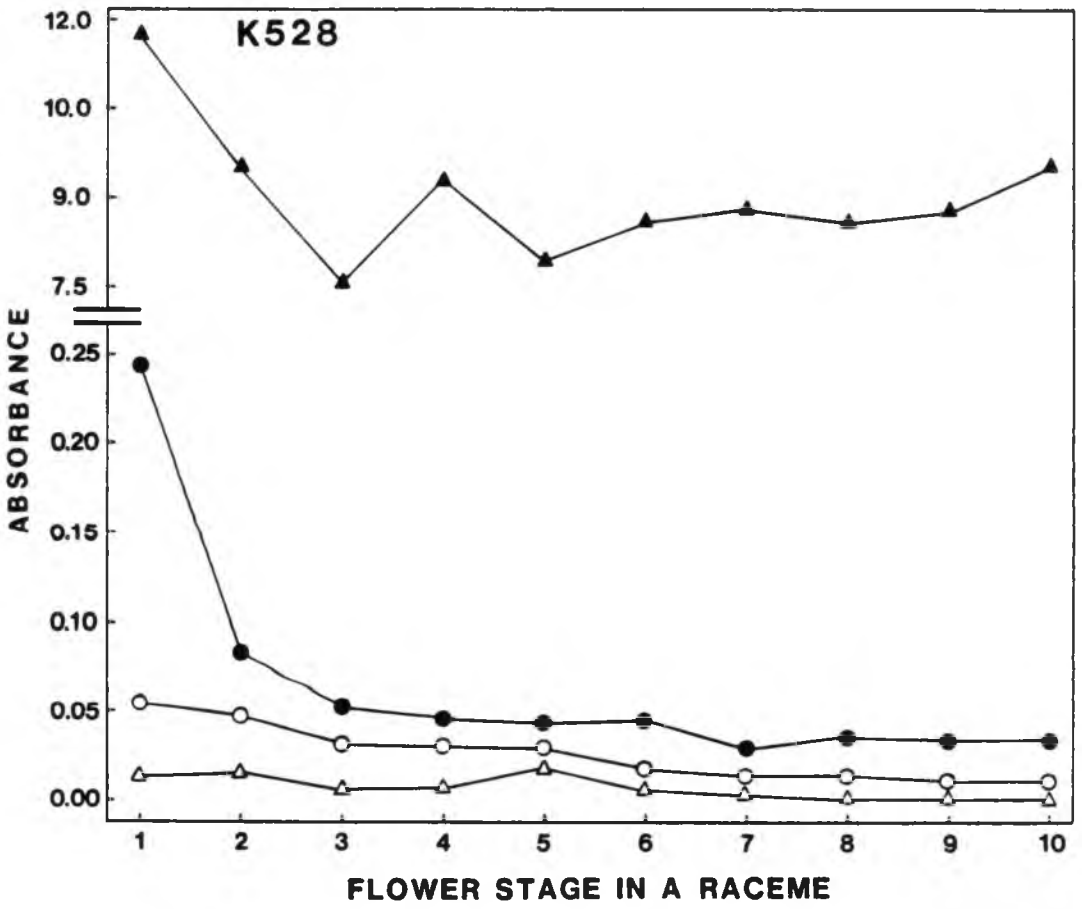


Figure 18. Absorbance of anthocyanins (△——△), flavonols (▲——▲), carotenoids (●——●), and chlorophylls (○——○) in K528 at different stages of flowers in a single raceme. Flower stage was numbered from the flower that had just opened as stage 1 followed down toward the raceme base to stage 10, respectively.

Figure 19. Fading of flower color in K637.

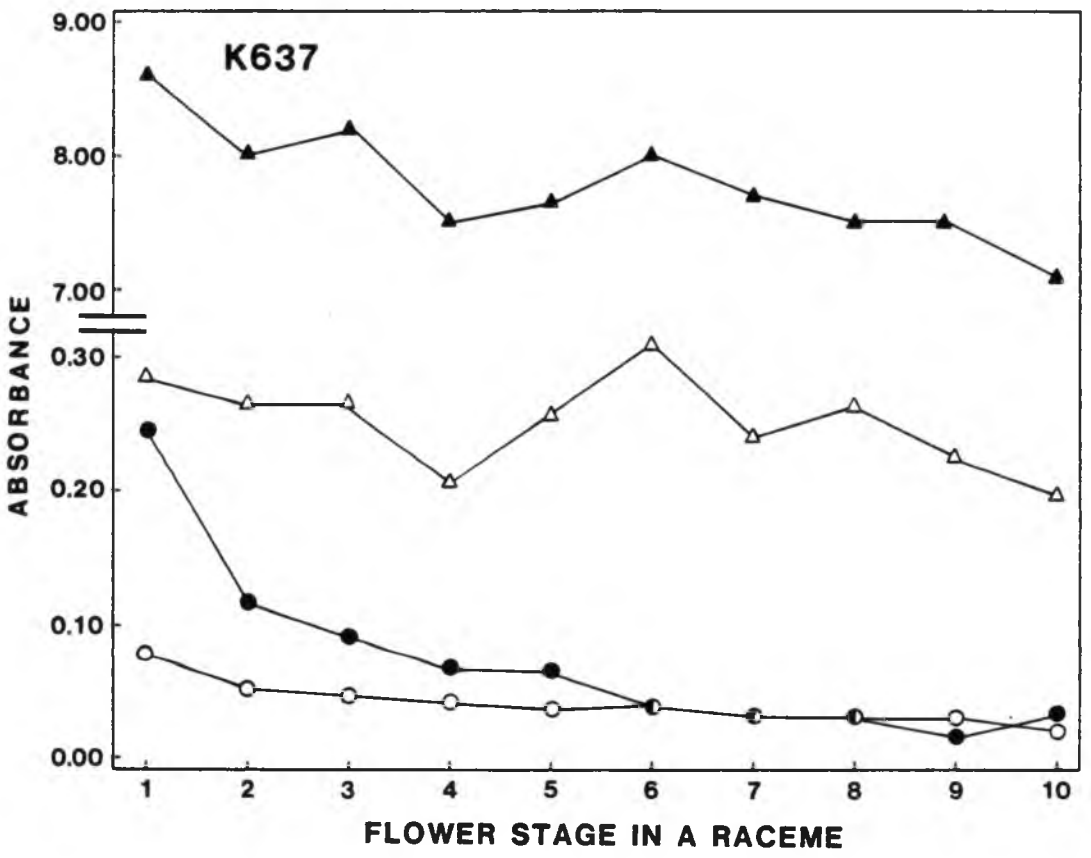


Figure 20. Absorbance of anthocyanins (△——△), flavonols (▲——▲), carotenoids (●——●), and chlorophylls (○——○) in K637 at different stages of flowers in a single raceme. Flower stage was numbered from the flower that had just opened as stage 1 followed down toward the raceme base to stage 10, respectively.

Figure 21. Flowers of K650.

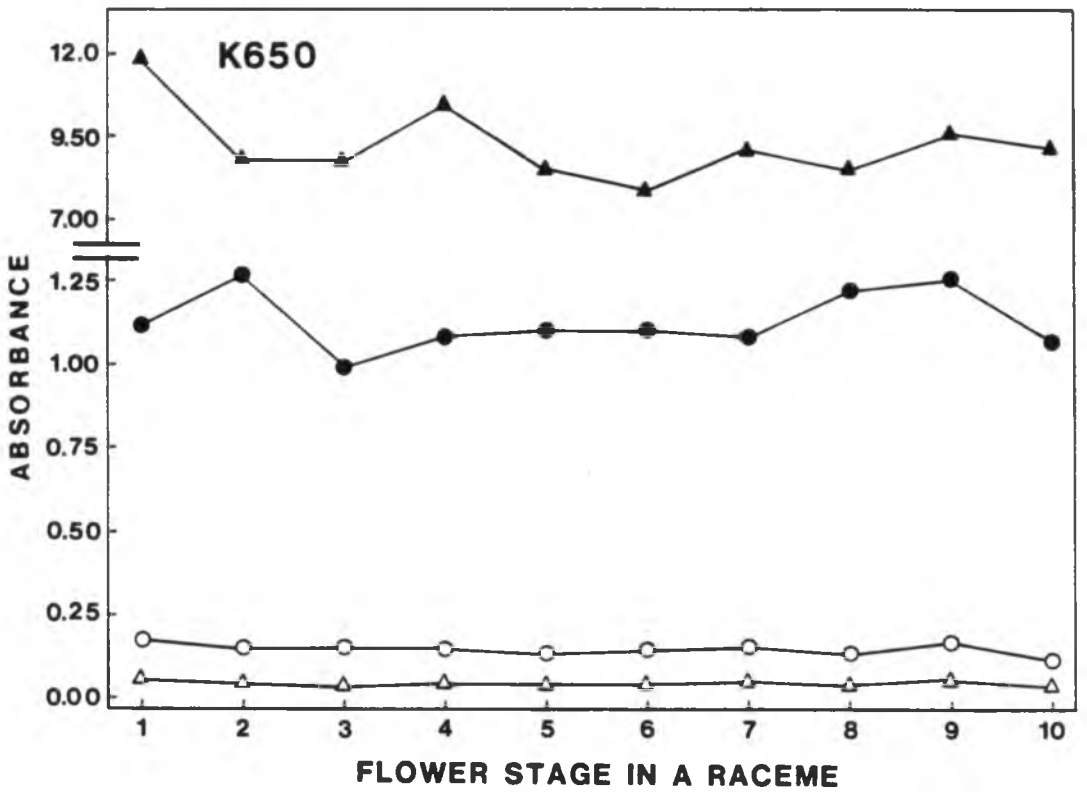


Figure 22. Absorbance of anthocyanins (Δ——Δ), flavonols (▲——▲), carotenoids (●——●), and chlorophylls (○——○) in K650 at different stages of flowers in a single raceme. Flower stage was numbered from the flower that had just opened as stage 1 followed down toward the raceme base to stage 10, respectively.

relatively stable. Chlorophylls decreased gradually. Flavonol content fluctuated and did not show any pattern of increase or decrease.

In K637 (Table 10; Figure 20), the absorbance of anthocyanins which contribute to purple coloration, was relatively high from stages 1 to 10, although there was a gradual decline. Carotenoids decreased rapidly from stages 1 to 4, then decreased gradually to very low amounts. Chlorophylls showed a slight decrease from stages 1 to 2, then maintained low concentrations through all stages. Flavonol content showed slight variation among the 10 stages.

In K650 (Table 10; Figure 22), carotenoids were found in the highest concentrations, compared to the other two crosses, and remained relatively constant. Anthocyanin content was low. All pigments, i.e. anthocyanins, flavonols, carotenoids, and chlorophylls, maintained their concentrations from stage 1 to 10.

4.2.2 Carotenoid and Chlorophyll Changes in Growth and Development of Flowers

Table 11 shows carotenoid and chlorophyll changes in growth and development of flowers of K528 and K637. At the bud stage of both crosses, 8 pigments were found in high concentrations, and their concentrations decreased when buds

Table 11. Quantitative distribution of carotenoids and chlorophylls at different stages of growth and development of flowers in two Dendrobium crosses.

Cross	Flower stage	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-carotene	Chl. a	Chl. b
		(μg/g fresh wt)							
K528	Bud(2-3.2cm)	6.2	4.5	3.8	17.3	2.1	34.0	64.1	34.2
	Blooming	1.8	1.4	1.4	4.2	0.6	5.8	14.2	8.9
	2-4 wks after blooming	0.2	0.1	0.3	0.9	0.4	-	-	-
K637	Bud(2-3.2cm)	5.3	3.4	3.3	14.1	1.6	23.1	56.6	27.2
	Blooming	3.7	2.6	3.2	8.2	1.6	15.7	31.5	17.6
	2-4 wks after blooming	0.2	0.1	0.1	0.1	0.1	-	1.0	0.2

developed to the blooming stage. Two to four weeks after blooming, β-carotene and chlorophyll a and b were not detected in K528 (Figure 23; Table 11) while only β-carotene was not detected in K637 (Figure 24; Table 11). In addition, other pigments that remained in the petals had low concentrations compared to the bud stage and the blooming stage.

4.2.3 Discussion

The three progenies, K528, K637, and K650, are relatively uniform because the parents involved were amphidiploids, near amphidiploid, and a species (Kamemoto, 1980). All three crosses had D. May Neal 'Srisobhon', an induced tetraploid, as one parent. Although D. May Neal 'Srisobhon' is a second generation hybrid, chromosome doubling has resulted in duplication of chromosomes and homozygosity in chromosome pairs. This plant has bright yellow petals and sepals, and contrasting purple lip. It has been widely used by orchid hybridizers to produce yellow-flowered offspring. K528 is a cross between D. May Neal 'Srisobhon' and amphidiploid D. Caesar with light yellow petals and purple in the center, but flower color faded to white rapidly. K637 is a cross between D. May Neal 'Srisobhon' and amphidiploid D. Jaquelyn Thomas '66217' with light purple flowers. K650 is a cross between D. May Neal 'Srisobhon' and D. helix with bright yellow petals.

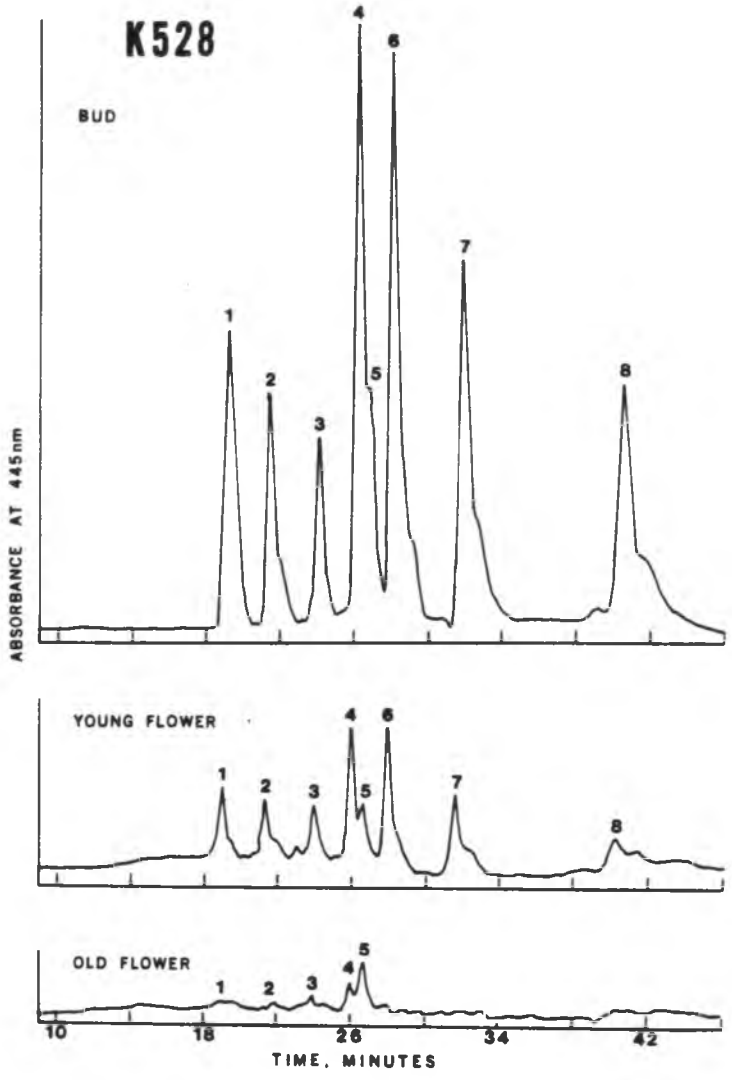


Figure 23. HPLC chromatogram of carotenoids and chlorophylls in different stages of growth and development in petals of K528. Bud, young flower, and old flower refer to 2.0-3.2 cm bud length, blooming, and 2-4 wks after blooming, respectively. Peak 1 = neoxanthin, Peak 2 = violaxanthin, Peak 3 = antheraxanthin, Peak 4 = lutein, Peak 5 = zeaxanthin, Peak 6 = chlorophyll b, Peak 7 = chlorophyll a, Peak 8 = β-carotene.

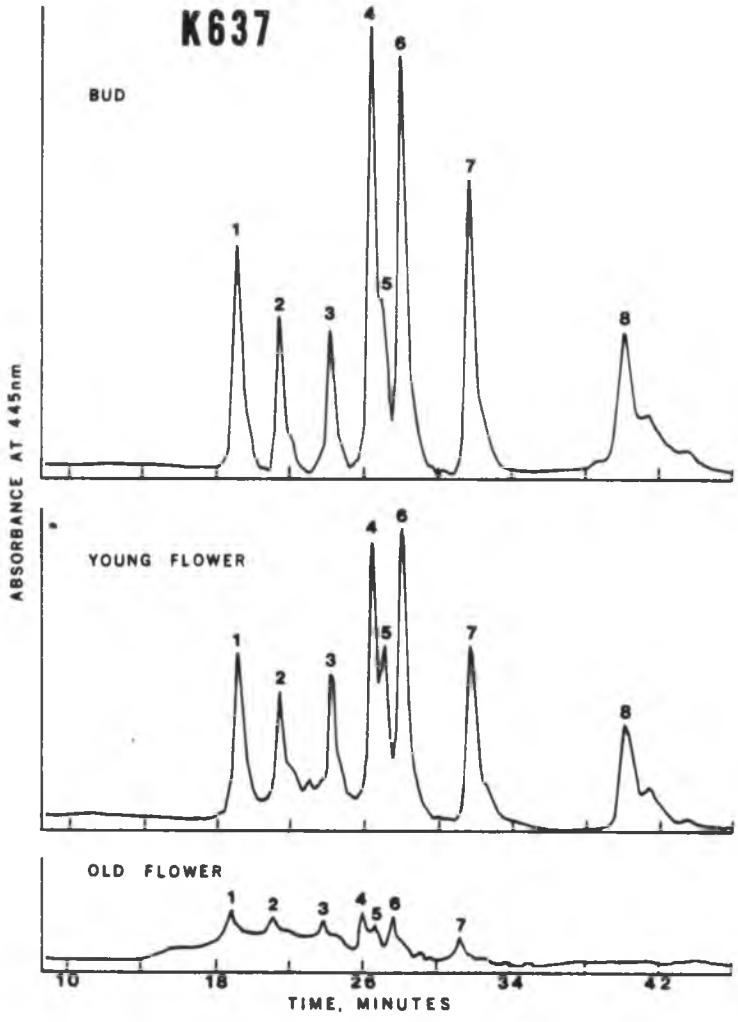


Figure 24. HPLC chromatogram of carotenoids and chlorophylls in different stages of growth and development in petals of K637. Bud, young flower, and old flower refer to 2.0-3.2 cm bud length, blooming, and 2-4 wks after blooming, respectively. Peak 1 = neoxanthin, Peak 2 = violaxanthin, Peak 3 = antheraxanthin, Peak 4 = lutein, Peak 5 = zeaxanthin, Peak 6 = chlorophyll b, Peak 7 = chlorophyll a, Peak 8 = β-carotene.

K528 and K637 are hybrids from one parent that has bright yellow petals (D. May Neal 'Srisobhon') and the other with light purple (D. Jaquelyn Thomas '66217') or creamish yellow petals fading to white (D. Caesar). The flower color of both crosses is pale yellow and fades rapidly after blooming. These two crosses suggest that yellow flower color is inherited quantitatively. Moreover, both crosses involve tetraploids, further complicating the nature of color inheritance. In contrast, K650, a triploid hybrid from two parents with bright yellow petals, did not show any fading of color. Thus, degradation might be due to genetic control because, among the three crosses examined, two out of three showed degradation.

There is no significant change of flavonols, which are co-pigments, in petals of the 3 crosses. This result is similar to that obtained by Pecket (1966). He observed color changes in flowers of Lathyrus hirsutus during senescence and concluded that the anthocyanin content decreased, but flavonol glycoside did not change in quantity.

Carotenoids and chlorophylls declined continually from bud stage to 2 weeks after blooming in K528 and K637 (Table 11). Stickland (1972) found similar results in florets of chrysanthemums. According to Valadon and Mummery (1969), epoxy-carotenoids and their derivatives in roses increased

and β-carotene decreased with age by oxidative processes. However, in the present study, neoxanthin, violaxanthin, and antheraxanthin, which are epoxy-carotenoids, decreased drastically in crosses K528 and K637 (Table 11).

4.3 Cytology and Yellow Flower Color Inheritance

4.3.1 Cytology

Chromosome numbers of some Dendrobium accessions are reported in Table 12. Most of these plants were of exceptional horticultural qualities sold at garden shops in Honolulu. Among sixteen plants cytologically examined, only two were diploids with 2n=38, while the rest were either triploids or tetraploids. Thus, the majority of improved yellow to bronze plants are polyploids.

4.3.2 Yellow Flower Color Inheritance

D. Jaquelyn Thomas 'Y166-1' is an amphidiploid which originated from chromosome doubling of a cross between D. phalaenopsis 'Lyon's Light No. 1', which is white with pinkish tinge on the abaxial petal and sepal surfaces, and a diploid white D. gouldii. Two generations of selfing and selection has resulted in K159-21 which was larger and whiter than 'Y166-1' (Figure 25).

Crosses were made between progeny of 'Y166-1' and the

Table 12. Chromosome numbers in some *Dendrobium* accessions.

Plant no.	Plant name	Flower color	Chromosome number (2n)
D186-1	*D.* Betty Ho 'Waimea'	yellow	57
D193	*D.* Spellbound	white with purple lip	38
D194	*D.* Spellbound	white with purple lip	76
D203-1,2	*D.* Spellbound	white with purple lip	38
D204-1	*D.* Joyce Uehara X (*D.* Shibata X *D.* Hula Girl)	yellow	57
D210-2	*D.* Ukio	yellow	57
D213-2	*D.* Floy Day 'Susan'	yellow	57
D216-1,2	*D.* Imelda Romualdez	yellow-bronze	76
D222-1,2	*D.* Myron Mooney	white	76
D234-1	*D.* C. K. Ai 'Oka'	yellow-bronze	57
D235-1,3	*D.* Tay Swee Keng	purple	76
D244	*D.* Mary Mak	yellow	57
D245	*D.* Field King AM/AOS	yellow	57
D254-1,2	*D.* Prince Kuhio X *D.* Liholiho	yellow	57
D269	*D.* Alice Queen	yellow	38
D270	*D.* *undulatum* var. *broomfieldii* 'Shimonishi'	yellow	57

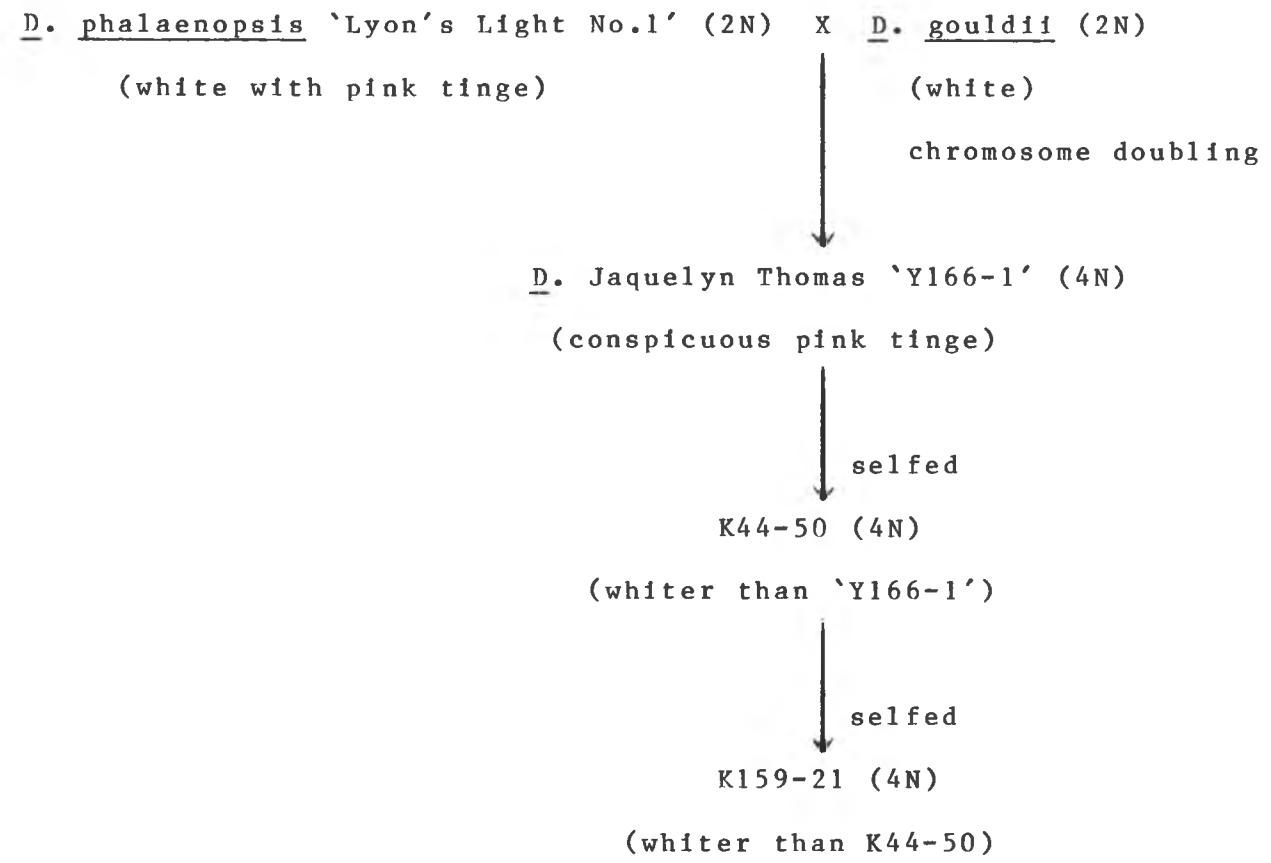


Figure 25. Relationships of D. Jaquelyn Thomas plants involved in improving yellow Dendrobium cultivars.

bright yellow D. May Neal 'Srisobhon', or the yellow-bronze D. Field King to possibly combine the high yields and other cut flower qualities of D. Jaquelyn Thomas with yellow flower color. Table 13 shows the segregation observed in various crosses in this program. Progeny K250 (K44-50 X D. Field King) were all light purple or purple although when the flowers first opened a creamish yellow which soon faded was observed. Progeny K382 (K159-21 X D. Field King) was mostly light purple and purple but a few plants were light yellow and one plant was light bronze. Progeny K500 (K250-29 selfed) segregated into light purple, light yellow, yellow, and white, recovering both the white and yellow colors of the parents. Progeny K487 (K250-29 X D. May Neal) and K526 (K250-29 X D. Field King) both segregated a range of flower colors from purple through shades of yellow and bronze to white, but K487 gave a higher proportion of yellow plants, indicating more transmission of genes for yellow by D. May Neal. Progeny K642 (D. Spellbound X a light lavender K382-18) also segregated into a range of purple, yellow, and white colors (Figure 26). Although D.Spellbound has white petals and sepals and a purple lip, it is a hybrid between D. Valley King and D. Pakanu, the second of which is yellow. Progeny K662, however, a cross between the white K159-21 and a yellow plant of progeny K487 (K487-131) gave all white progeny. However, some plants showed a light

Table 13. Segregation of offspring of crosses which have D. Jaquelyn Thomas 'Y166-1' as an ancestor.

Cross no.	Parents	Progeny phenotype[Z]							
		LP	P	LY	Y	LBR	BR	W	Total
K250	K44-50 X D. Field king (white) (yellow-bronze)	29	3	-	-	-	-	-	32
K382	K159-21 X D. Field King (white) (yellow-bronze)	8	8	4	-	1	-	-	21
K500	K250-29 selfed (light purple)	11	-	4	2	-	-	3	20
K487	K250-29 X D. May Neal (LP) (yellow)	2	-	21	36	6	8	7	80
K526	K250-29 X D. Field King (LP) (yellow-bronze)	7	-	4	1	3	3	-	18
K642	D. Spellbound X K382-18 (white) (light lavender)	2	19	13	17	-	-	15	66
K662	K159-21 X K487-131 (white) (yellow)	-	-	-	-	-	-	20	20

[Z]LP = light purple, P = purple, LY = light yellow, Y = yellow, LBR = light bronze, BR = bronze, W = white.

Figure 26. Flower color segregation in K642.

yellow color when the flowers first opened which soon faded.

Table 14 shows the quantitative distribution of carotenoids and chlorophylls in the petals of some Dendrobium crosses along with their parents. The offspring of K432, K528, K650, and K751 were relatively uniform in flower color because parents involved are amphidiploid (D. Caesar), near amphidiploid (D. May Neal 'Srisobhon'), or species (D. strebloceras, D. helix, and D. canaliculatum). These parents when selfed or crossed produce uniform F_1 progeny (Kamemoto, 1980).

K432 flowers were greyed-orange, close to one of its parents, D. strebloceras. D. strebloceras had lutein, chlorophylls a and b, and some unknown carotenoids that had retention times between chlorophyll a and β-carotene (Figure 14). These unknown carotenoids had higher peaks than the identified compounds. The predominance of these unknown carotenoids infers that they contribute major color in D. strebloceras. K432, however, did not or had very small amounts of these unknown carotenoids, and so were not detected with HPLC. Moreover, K432 had all 8 major pigments, but in lower concentrations than the other parent, D. canaliculatum, except β-carotene (Table 14). Thus, all carotenoid pigments were transmitted from D. canaliculatum to the hybrid.

K528 had carotenoid and chlorophyll amounts higher than

Table 14. Quantitative distribution of carotenoids and chlorophylls in petals of some Dendrobium crosses with their parents.

Cross no.	Parent and offspring	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-carotene	Chl. a	Chl. b
		(μg/g fresh wt)							
K432	D. strebloceras (2N) (greyed orange) (168B)[Z]	-	-	-	53.4	-	-	154.5	24.1
	D. canaliculatum (2N) (yellow green) (154B)	10.2	11.6	5.9	26.4	2.7	105.3	86.2	41.7
	Offspring (greyed orange) (74D + 163A)	2.1	5.2	5.6	10.9	2.6	192.3	45.1	13.9
K528	D. Caesar (4N) (pale yellow) (155B)	0.2	0.2	0.3	0.8	0.1	-	4.0	1.5
	D. May Neal (4N) (yellow) (7A)	8.1	4.6	2.3	18.9	1.9	43.0	37.6	24.4
	Offspring (pale yellow) (150B)	1.8	1.4	1.4	4.2	0.6	5.8	14.2	8.9
K650	D. May Neal (4N) (yellow) (7A)	8.2	4.6	2.3	18.9	1.9	43.0	37.6	24.4
	D. helix (2N) (yellow) (7B)	12.6	5.6	4.8	32.3	3.7	4.7	26.1	23.0
	Offspring (yellow) (151B)	28.0	12.5	4.5	36.9	3.7	120.5	104.1	47.4
K751	D. Caesar (4N) (pale yellow) (155B)	0.2	0.2	0.3	0.8	0.1	-	4.0	1.5
	D. canaliculatum (2N) (yellow green) (154B)	10.2	11.6	5.9	26.4	2.7	105.3	86.2	41.7
	Offspring (pale yellow) (154D)	0.2	0.3	0.8	1.6	0.7	2.9	9.0	3.6

[Z]Flower color was matched with the color chart of the Royal Horticultural Society, London.

in D. Caesar but much lower than in D. May Neal 'Srisobhon' (Table 14). D. Caesar (D. phalaenopsis X D. stratiotes) had creamish yellow, which was passed on from D. stratiotes, but the light yellow color faded rapidly after flowers opened. This was similar to the color changes obtained in K528.

K650 had high concentrations of carotenoids and chlorophylls similar to both parents, D. May Neal 'Srisobhon' and D. helix (Table 14). This cross tended to contain higher pigment concentrations than its parents, especially for β-carotene, chlorophylls a and b.

K751 flowers were creamish yellow but the color faded rapidly after blooming. K751 and one of its parents, D. Caesar, had low concentrations of the pigments, while the other parent, D. canaliculatum, had high pigment concentrations (Table 14).

4.3.3 Discussion

Most of the Dendrobium accessions examined cytologically were award-winning orchids. The results showed that the majority are triploids and tetraploids (Table 12). Increase in ploidy will result in an increase in size of the flower parts, such as width and substance of the sepals and petals (Tanaka and Kamemoto, 1984), which are desirable characteristics. For example, a tetraploid D. Spellbound (D194) has larger flower width and heavier

substance than the diploid counterpart (D193). Therefore, many award-winning plants are triploids and tetraploids.

Relatively little research on color inheritance in orchids, especially in the genus Dendrobium, has been conducted. Dendrobiums are open-pollinated crops, have complex flower colors, and long life cycles of 4-5 years. In some dendrobiums, flower color of sepals, petals, and lips (modified petals) are completely distinct. Even in the same tissue, many colors are mixed together like an art work. Therefore, it is difficult to elucidate color inheritance in Dendrobium flowers. A methodology for carotenoid and chlorophyll identification was developed in this study. Further biochemical investigations might lead to a better understanding of the inheritance of yellow and green flower color.

Yellow Dendrobium flowers have various degrees of yellowness, such as yellow tinge in vascular tissue, creamish yellow, pale yellow, bright yellow, yellow-orange, and yellow-bronze. The color is not clearly classified. From the biochemical study of the pigments, 6 major carotenoids and chlorophylls a and b were detected in different quantities in the majority of yellow flowers. This may suggest that yellow color is a quantitative trait. In other words, carotenoids are controlled by several genes.

Carotenoid and flavonoid biosynthetic pathways are

unrelated; therefore, both groups of pigment are genetically independent (Harper, 1972b). Griesbach (1984) studied carotenoid-anthocyanin combinations on the bronze flowered hybrid, Phalaenopsis amboinensis X Doritaenopsis Grebe. Phalaenopsis amboinensis flowers are yellow with brown spots, which are due to carotenoids. Doritaenopsis Grebe flowers are magenta, which is due to a magenta anthocyanin. The bronze color in the Doritaenopsis hybrid is the combined effect of carotenoids and anthocyanins, which is inherited qualitatively. His results are different from other studies in the genera Oncidium, Phalaenopsis, and Dendrobium. In section Variegata of the genus Oncidium, yellow color is dominant over red color, masking the reds or confining the red color to the back of the flowers (Moir and Moir, 1980). Freed (1979) reported that usually the yellow colors fade or may be hidden in the first generation and will show in the following generation.

The results from the Dendrobium breeding program at the University of Hawaii showed that crosses between two bright yellow-flowered parents produce offspring that are yellow, and the color does not degrade rapidly. On the other hand, if one parent has flowers which are white, light purple, or yellow which fades rapidly, the offspring can be white or light purple with creamish or pale yellow, which fades rapidly.

Table 13 shows that K44-50 and K159-21 do not have any carotenoids. When they were crossed with yellow-bronze flowered D. Field King, the offspring (K250) had purple color or light purple with pale yellow which faded rapidly. Some segregation in flower color of K250 was probably due to the heterozygous nature of D. Field King (Kamemoto, 1980). Selfing a selected hybrid individual (K250-29) produced a segregating progeny (K500). Crossing the selected hybrid individual (K250-29) with a bright yellow flowered plant, either D. Field King or D. May Neal 'Srisobhon', produce a high proportion of yellow-flowered offspring. In K642 (D. Spellbound X K382-18)", the yellow flower color hidden in the parent generation was expressed in this progeny. Progeny of K662 (K159-21 X K487-131) gave all whites (although some showed light yellow when flowers opened which later turned white) because K159-21 was white and K487-131 was a hybrid between light purple (K250-29) and bright yellow (D. May Neal) parents; therefore, yellow color was hidden or did not have high color intensity. It is difficult to elucidate the yellow flower color inheritance from Table 13 because numbers of offspring observed are not sufficient and D. Field King has heterozygous genotype.

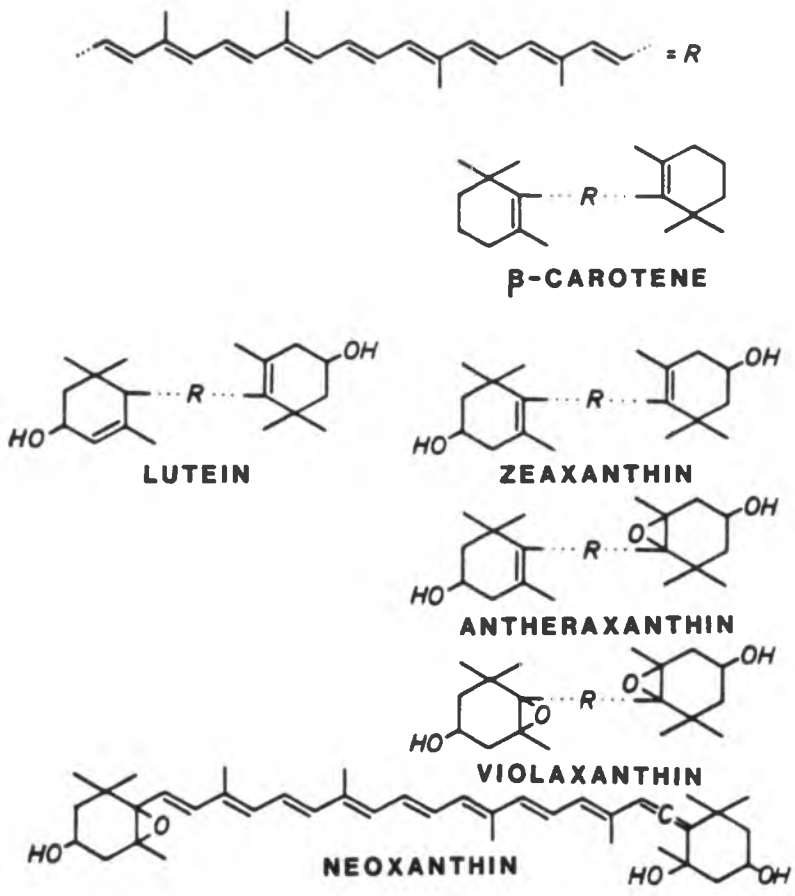
It is interesting to note that K650 is the hybrid from parents which have different ploidy levels (Table 14). K650 is a triploid progeny derived from the cross between diploid

D. helix and tetraploid D. May Neal 'Srisobhon' (Kamemoto et al., 1972). Unlike the progenies of K432, K528, and K751, the flowers of K650 have carotenoid and chlorophyll content close to or slightly higher than the 'Srisobhon'. This may be due to the effect of polyploidy.

K751 is also a triploid hybrid, but its flowers have relatively low concentrations of carotenoids and chlorophylls (Table 14). This triploid progeny inherited only one chromosome set from D. canaliculatum, which has high amounts of carotenoids and chlorophylls. The other two chromosome sets came from D. Caesar, which has low amounts of the pigments.

In general, carotenoids and chlorophylls are inherited quantitatively with additive effects of gene interaction since hybrids tend to have pigment concentrations ranging between those of both parents.

APPENDIX



CAROTENOIDS

Figure 27. Structure of carotenoids identified in yellow Dendrobium petals.

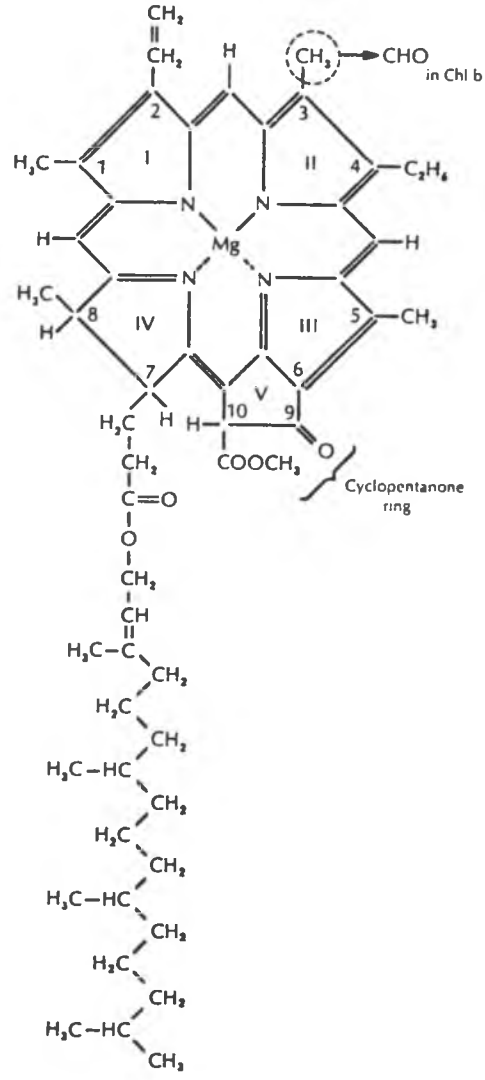
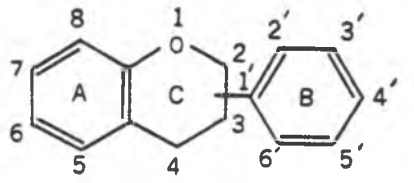
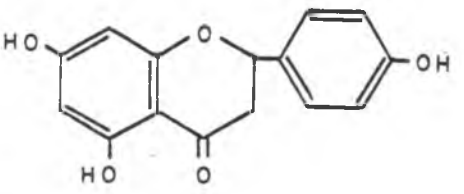


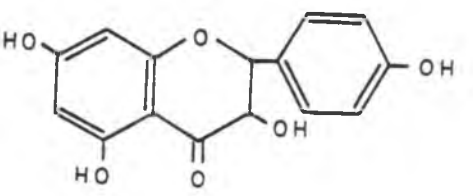
Figure 28. Structure of chlorophyll a and b. To form chlorophyll b an aldehyde group replaces the methyl group at the C_3 atom.



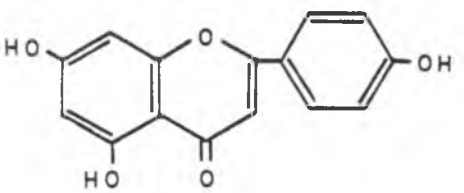
BASIC STRUCTURE



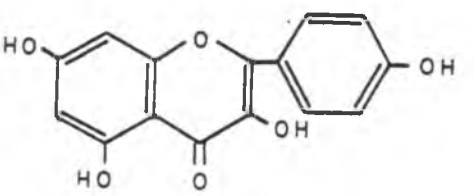
FLAVANONE



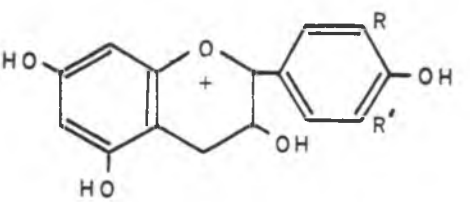
FLAVANONOL



FLAVONE



FLAVONOL



ANTHOCYANIDIN

Figure 29. Structure of common flavonoids contributing flower color.

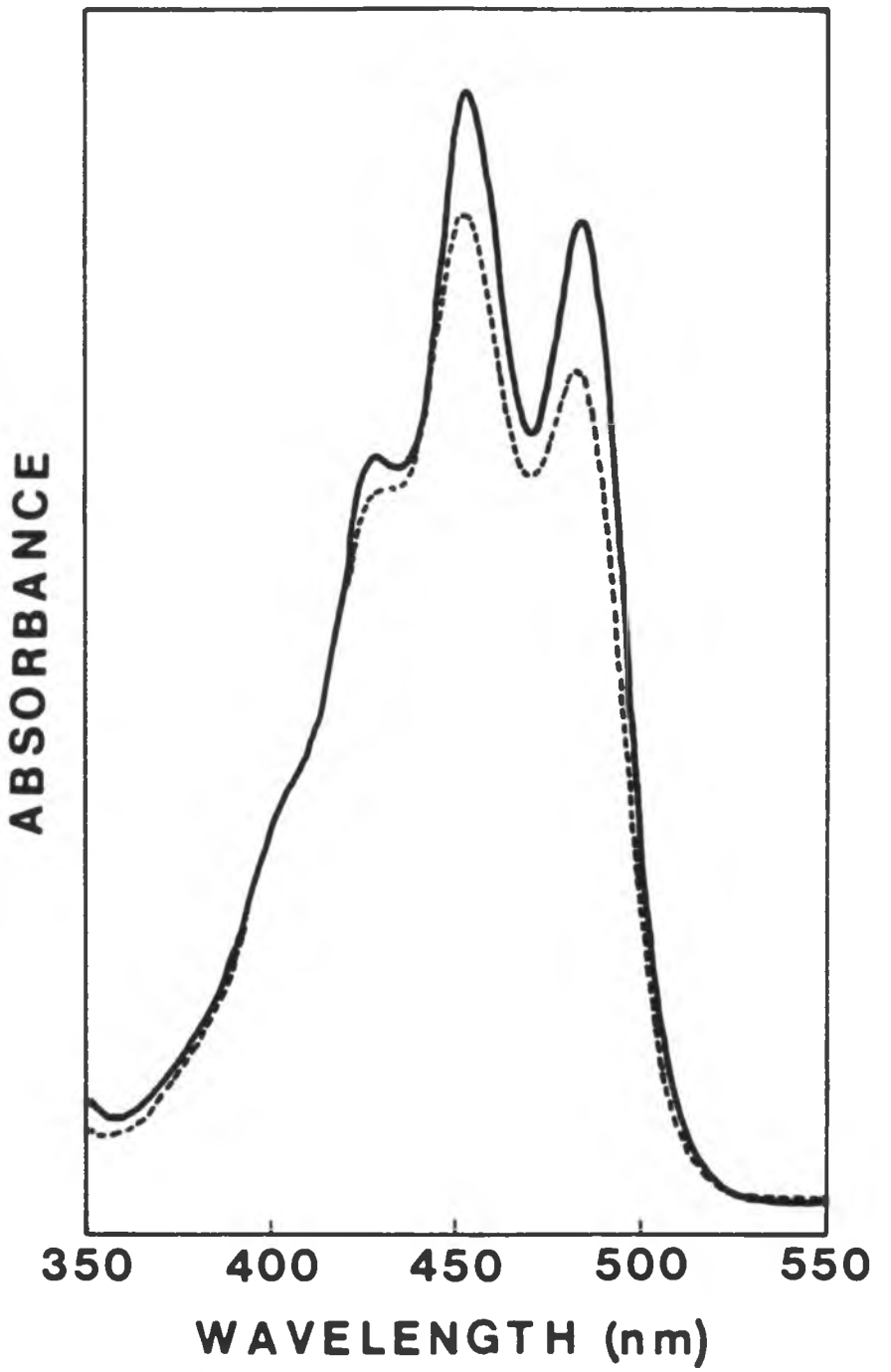


Figure 30. Absorption spectra of an unknown carotenoid (Peak A detected in D. strebloceras) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (---).

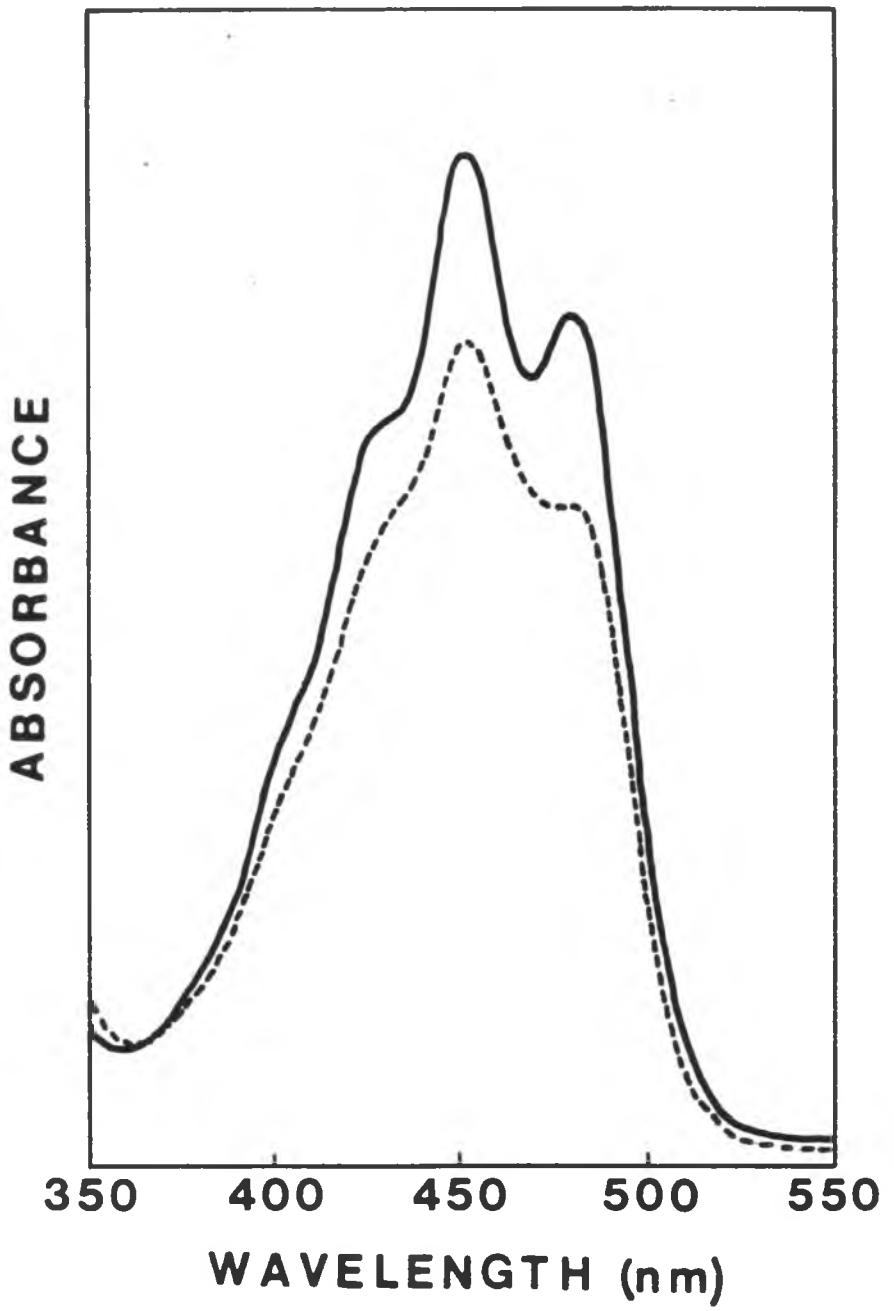


Figure 31. Absorption spectra of an unknown carotenoid (Peak B detected in D. strebloceras) in methanol/acetonitrile (25:75 V/V) (——) and with addition of HCl (---).

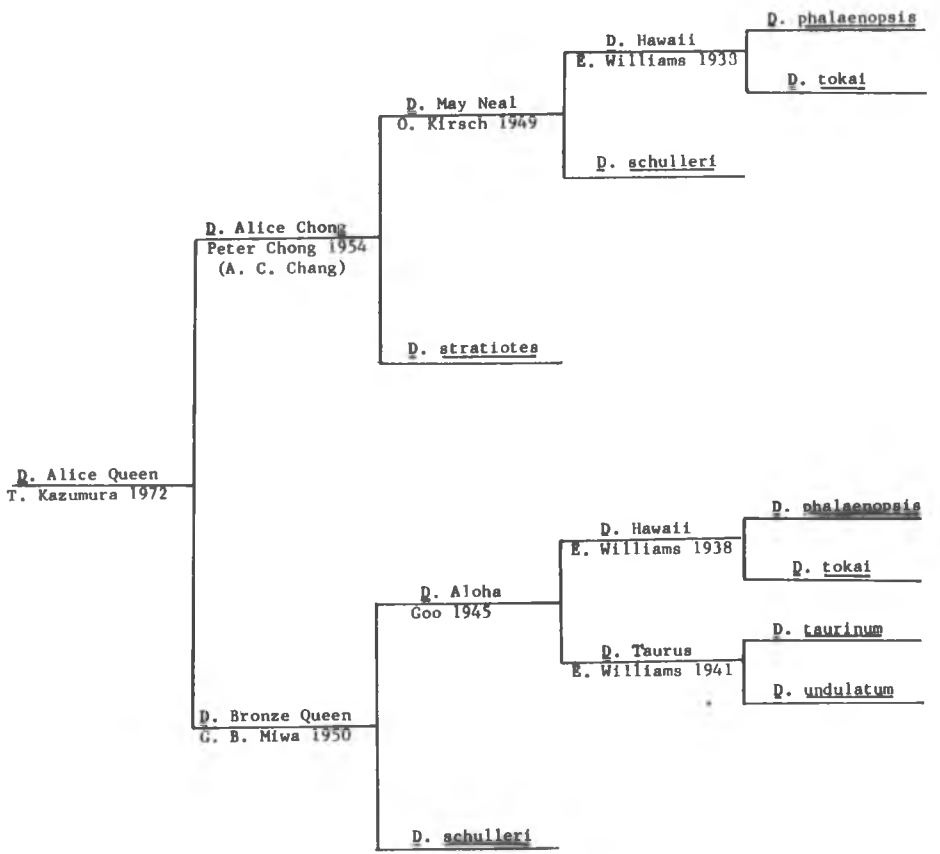


Figure 32. Pedigree of D. Alice Queen.

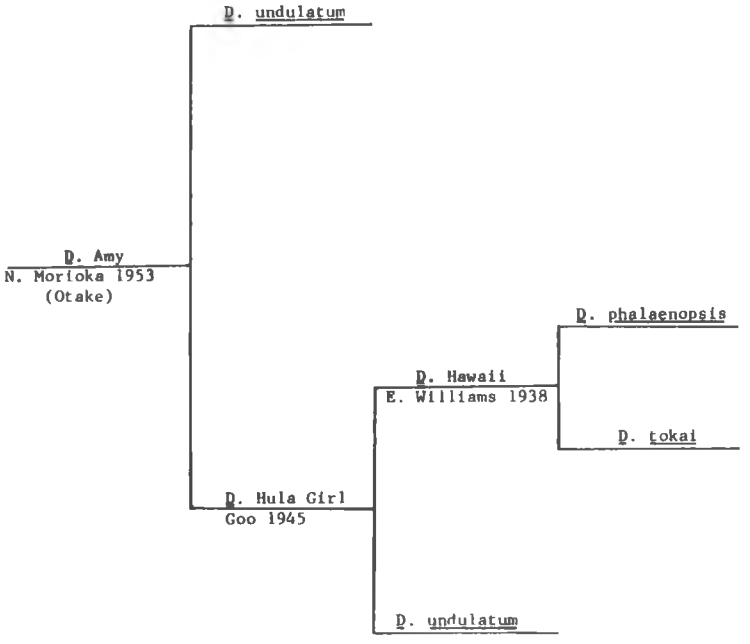


Figure 33. Pedigree of D. Amy.

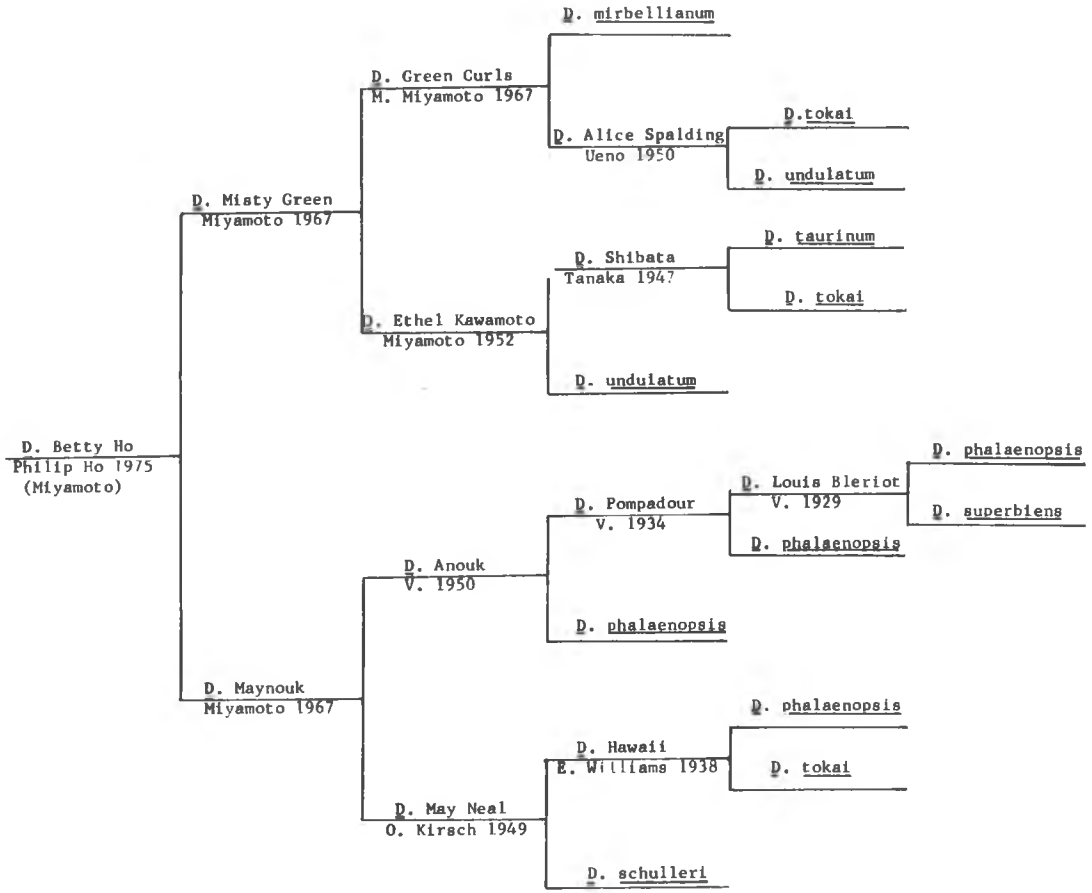


Figure 34. Pedigree of D. Betty Ho.

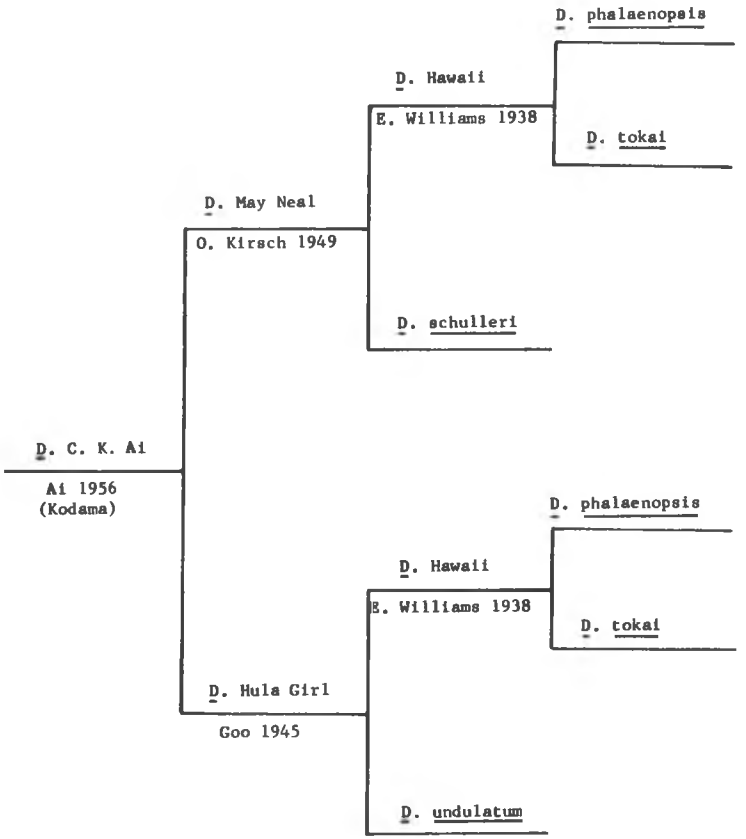


Figure 35. Pedigree of D. C. K. Ai.

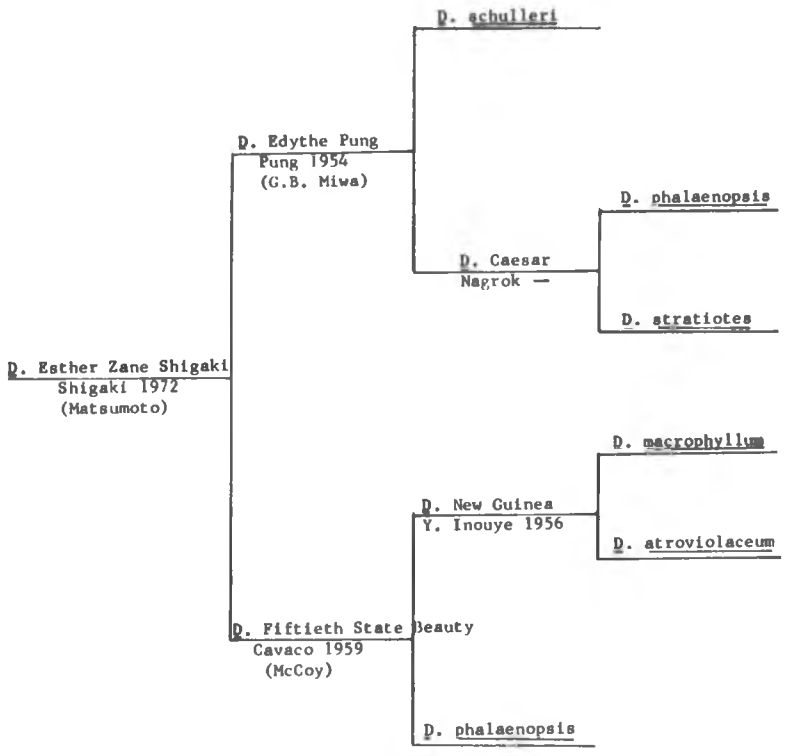


Figure 36. Pedigree of D. Esther Zane Shigaki.

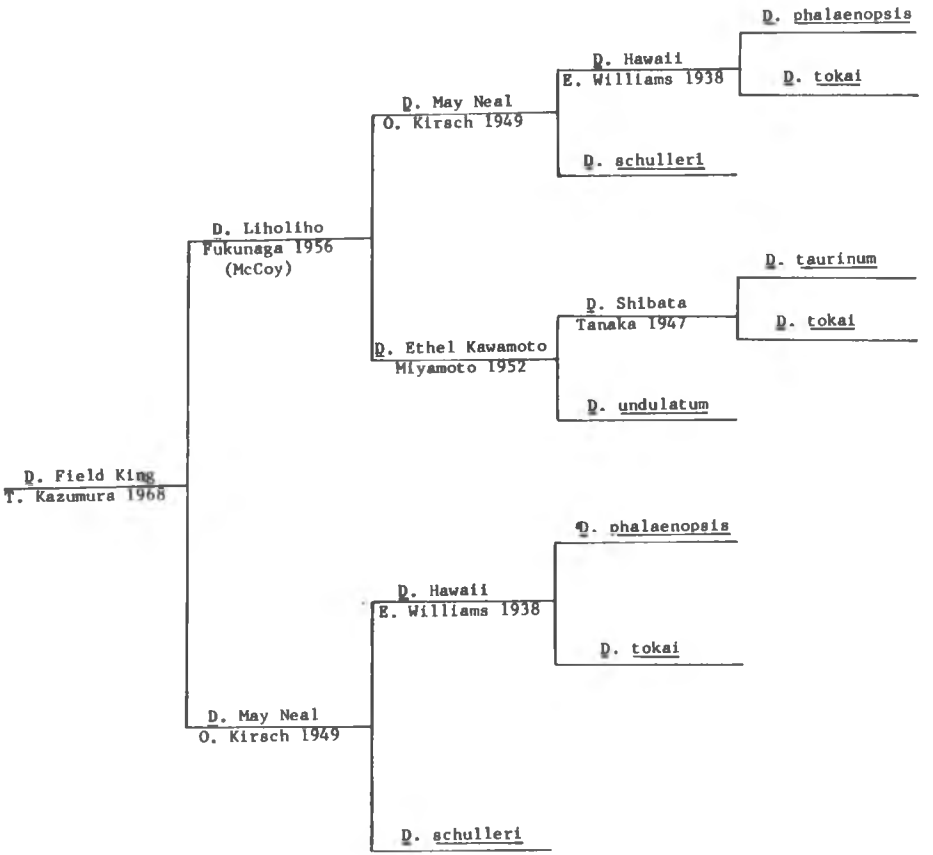


Figure 37. Pedigree of D. Field King.

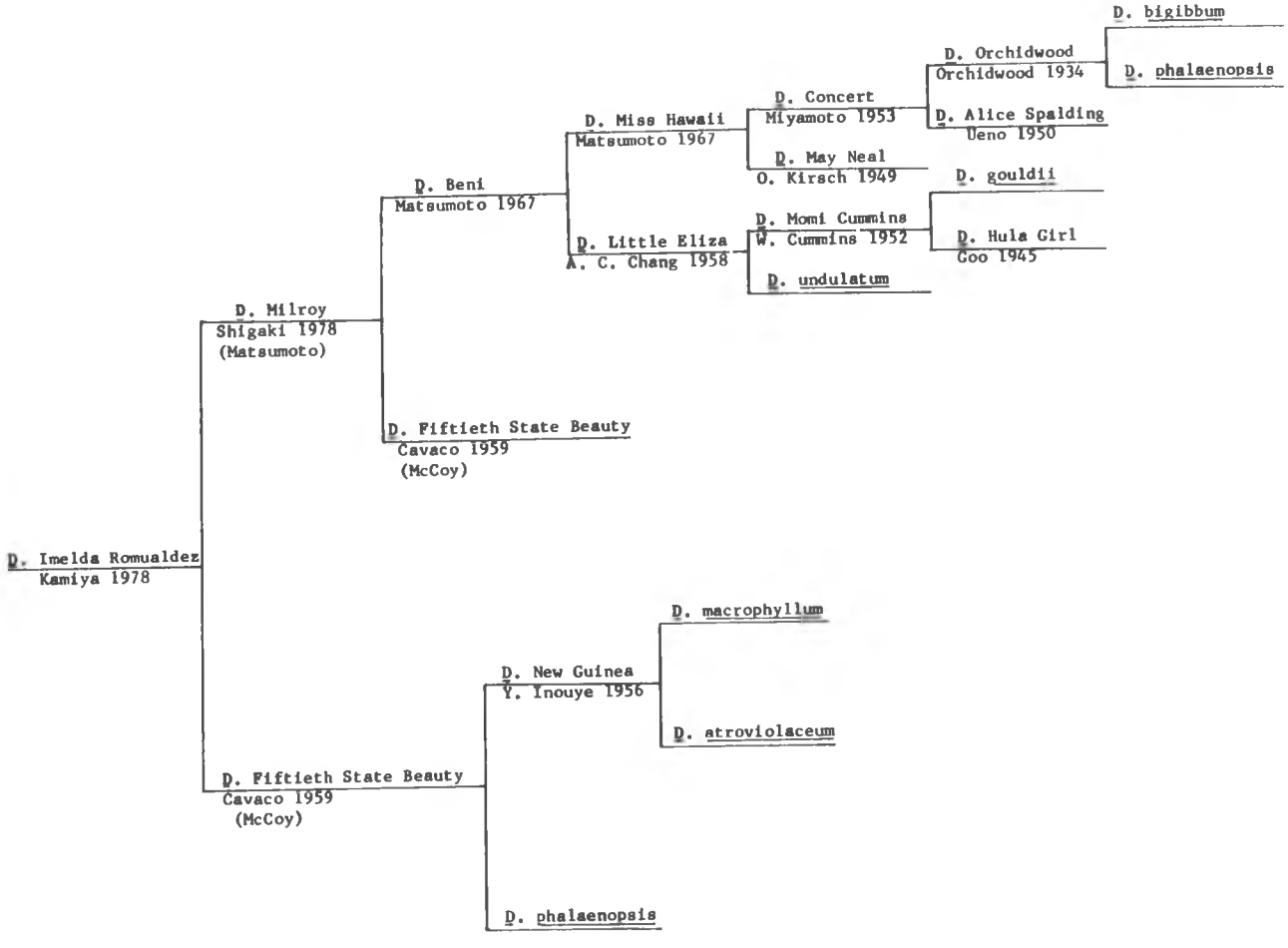


Figure 38. Pedigree of D. Imelda Romualdez.

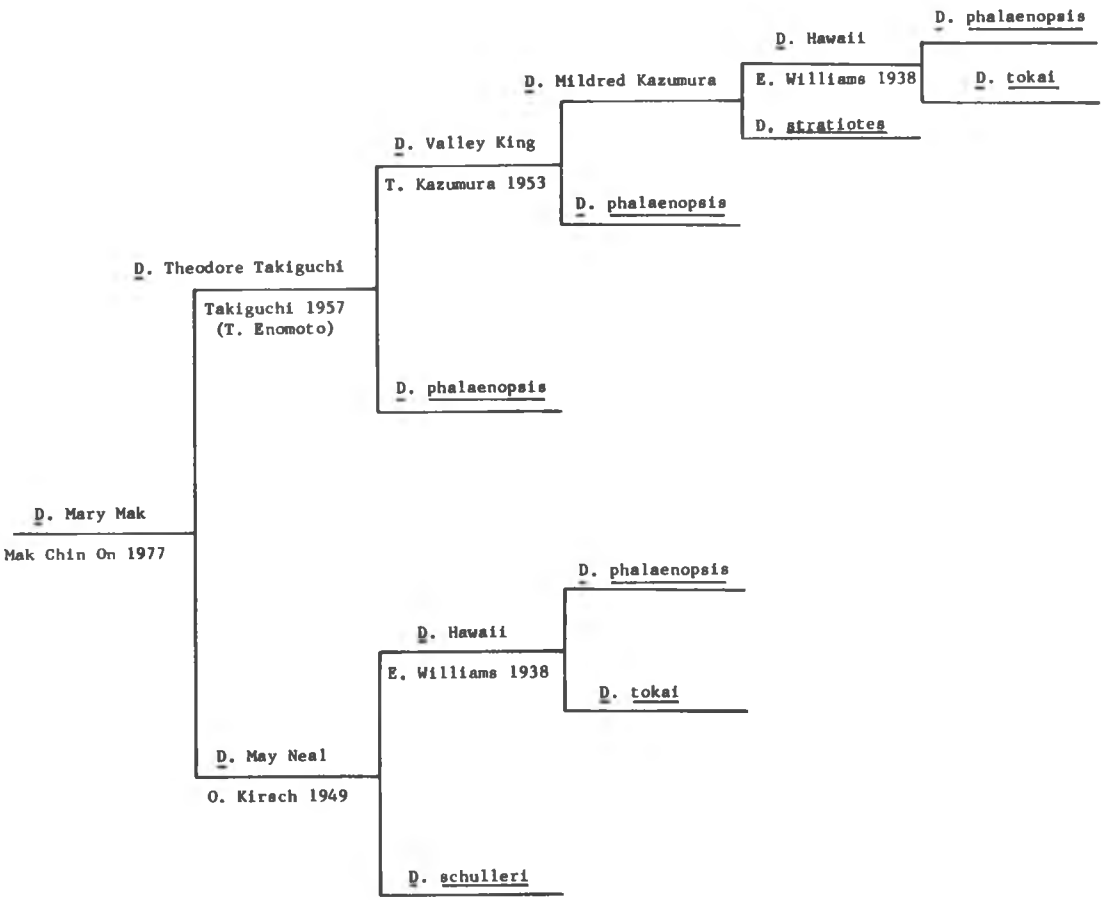


Figure 39. Pedigree of D. Mary Mak.

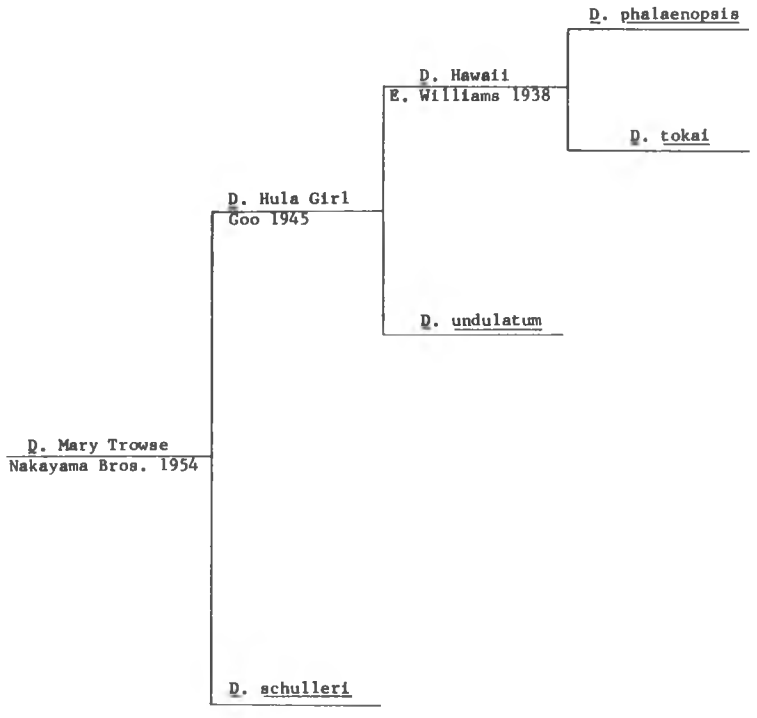


Figure 40. Pedigree of D. Mary Trowse.

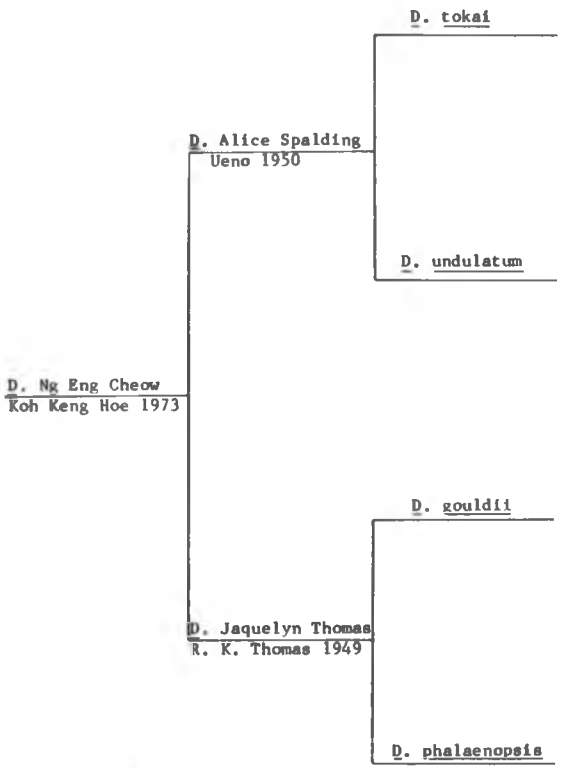


Figure 41. Pedigree of D. Ng Eng Cheow.

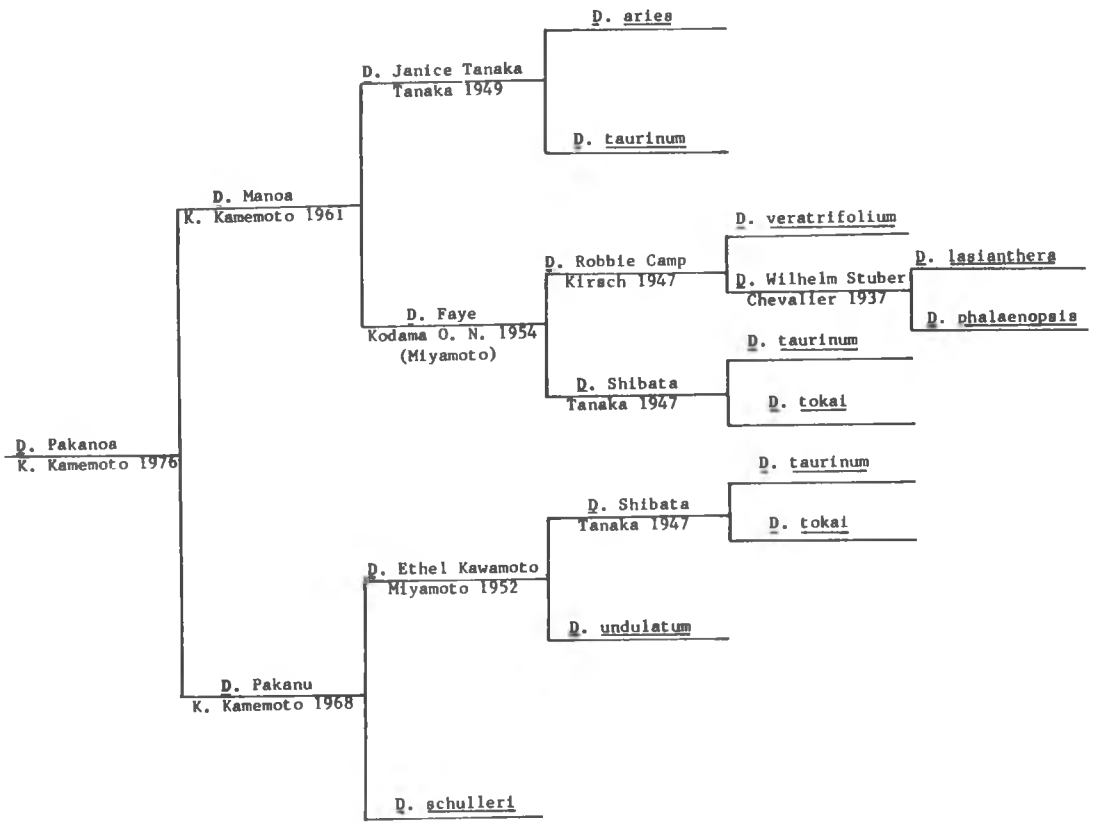


Figure 42. Pedigree of D. Pakanoa.

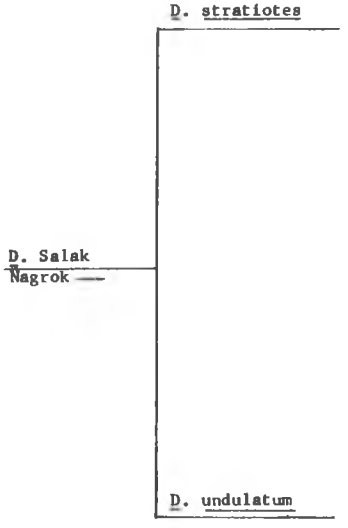


Figure 43. Pedigree of D. Salak.

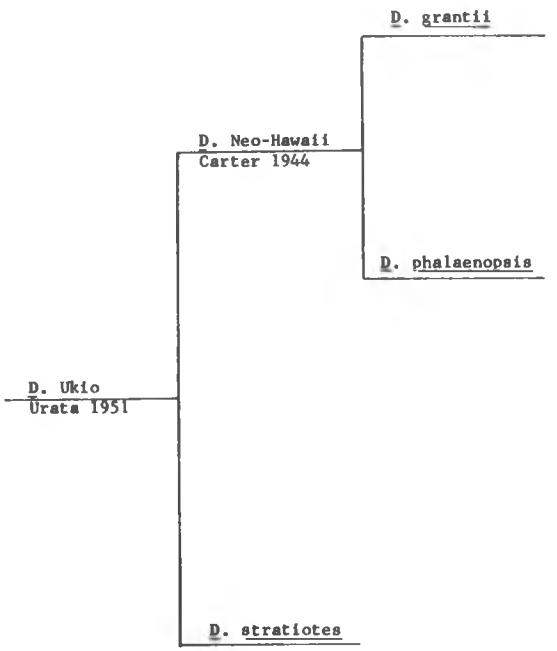


Figure 44. Pedigree of D. Ukio.

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